

## ON SOME TREMATODE-PARASITES OF AUSTRALIAN FROGS.

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(Plates xiv.-xliii.)

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### SECTION I.

*Introduction.*—Swammerdam seems to have made the first recorded observation of a trematode living as a parasite in frogs, when he mentions, in his “*Biblia naturæ s. historia animalium, etc.*,” in 1737, a worm from the lung of a frog. This worm was not named, however, till 1800, when Zeder(103) described and named it *Distomum cylindraceum*. In the intervening time, it had been met with by Pallas(74) and Goeze(21), both of whom failed to definitely recognise it, the former putting it down as *Fasciola subclavata*, the latter as *Planaria cylindrica*. This worm, now known as *Haplometra cylindracea*, is not only the first trematode obtained from frogs, but enjoys the distinction of being one of the oldest known trematodes.

Then in 1758, Roesel von Rosenhof(78) described and figured a trematode from the bladder of a frog without, apparently, naming it; Zeder(103) called it *Polystoma ranæ*, and Rudolphi (79, p.451) described and figured it as *Polystoma integerrimum*.

Loschge(56), in 1785, published in "Naturforscher" a description and figure of a trematode from the bladder of the green water-frog, which Zeder(103) named *Distomum cygnoides*. In 1787, Goeze(21) described *Diplodiscus subclavatus* as *Planaria subclavata*.

The next frog-trematode to appear in literature is *Opisthio-glyphe endoloba*, which Froelich(20), in 1792, included with *Diplodiscus subclavatus* under the name *Fasciola ranæ*. Rudolphi referred Froelich's *Fasciola ranæ* to his own *Distomum clavigerum*, and, in this, was followed by a number of later writers, e.g., Dujardin and Diesing. Dujardin found the real *O. endoloba*, however, and recognising it as distinct from *D. clavigerum*, described it, in 1845(18), as *Distomum endolobum*.

*Pneumonæces variegatus* was known to Zeder, who, however, failing to perceive its ventral sucker, classed it as a *Monostomum*, describing it(103) as *Monostoma bombynæ*. Rudolphi(79) also at first failed to detect the ventral sucker, and changed Zeder's species-name into *ellipticum*. Later(80), recognising the ventral sucker in some worms from a similar source, he described it as *Distomum variegatum*.

In his "Entozoorum . . . historia naturalis," the most important work on Entozoa up to that time, Rudolphi gave a description of six species of trematodes from frogs—*M. ellipticum*, *A. subclavatum*, *D. cygnoides*, *D. crassicolle*(from the Salamander), *D. cylindraceum* and *P. integerrimum*; while in 1819(80) he added *D. variegatum* and *D. clavigerum*. Diesing, in his comprehensive work(17), gave a description of all these, as well as *D. endolobum* and *D. retusum*.

In 1860, Vulpian(100) described *Halipegus ovocaudatus*, from the buccal cavity of frogs, as *Distomum ovocaudatum*. Olsson(70), in 1878, described, as new, three frog-trematodes—*D. vitellilobum*, *D. rastellus* and *D. medians*, the last of which alone has proved to be an independent species.

Sixteen years later, Looss published his fine work(48) on the Distomidæ of Fishes and Frogs, giving very complete accounts of the anatomy of eight species of trematodes from frogs, and defin-

itely separating *D. clavigerum*, *D. medians* and *D. confusum*. This was followed several years later by those two magnificent works of Looss, the "Versuch"(50), and the "Trematodes of Turtles, etc."(55), and with these a new era in the systematic history of the Malacocotylea began.

Up to this time, the modern views as to what constituted specific and generic, and even subfamily and family characters of these worms, had scarcely begun to make their appearance, and the detailed classification of the group was in a very unsatisfactory state. A very comprehensive account of the Trematodes, in regard to anatomy and life-history, covering practically all that was known of them up to that time, had been given, indeed, by Braun in his admirable and monumental volume on this Class in Bronn's "Classen u. Ordnungen, etc."(4), but the dawning of our modern conceptions regarding the definition of the species and genera was not foreshadowed in this work. The genus *Distomum* Retz., comprised at this time what are now looked upon as a number of separate families, including a very large number of genera. Many of the old species (*e.g.*, *D. variegatum* and *D. cygnoides*) have since been shown to have really comprised a number of species and in some cases even several genera. Owing to the real specific differences and the limitations of the natural genera of these worms not having been recognised, a natural classification of the distomes had not been able to be propounded. Looss points out(55, p.809) as a result of this want of recognition of the real specific characters ". . . dass verschiedene Species, die meistens derselben natürlichen Gattung angehören, früher in eine einzige zusammengeworfen, und dass die betreffenden natürlichen Gattungen damit auf das Niveau blöser Species herabgedrückt worden sind."

When trematodes from frogs, for instance, were first recorded from America, they were referred to the European species to which they appeared to be most closely related, *e.g.* Wright(102). Later, some became distinguished as special varieties, *e.g.* Bensley(3) and later still, when the meaning of the small but constant differences in structure which they exhibited, came to be realised, they were described as independent species.

One of the difficulties in the way of the formation of natural genera, as "muster" groups of species, was the large number of species standing isolated without any apparent generic companions. One of the chief reasons for the apparent isolation of such species was, of course, as so clearly declared by Looss, that the species in question was not a real species, but a whole group of species, the differentiating characters of which had escaped observation; but, in many cases at least, the reason seems to me to have been that their generic companions had not yet been discovered. This difficulty is now being rapidly overcome by the discovery and description of trematodes from, for the most part, similar sources in other zoogeographical regions. On this subject, I shall have more to say in a later section of this work. The Trematodes are a very old group of animals and, owing to the conditions under which their parasitic life is passed, they experience evolutionary changes much more slowly than their hosts, so that we find closely related species of the same genus of these worms living in hosts which are not only now widely separated geographically, but which have diverged from their parent-stock even beyond the limits of natural genera.

The present-day, more satisfactory state of our knowledge of the classification of the Malacocotylea owes much to the recent work of Looss, Braun, Lühe and Odhner, who, within the last few years, have defined many natural genera, subfamilies, and families of this group.

The Trematodes of American frogs have been described, and their relationships pointed out chiefly by Stafford(85-91), and Pratt(75-77), and to a less extent by Bensley(3), Nickerson(66), and Seeley(81); while the Asiatic forms have been investigated by Lühe(60), and Klein(38).

#### SECTION II.

*Material Investigated.*—The first Trematodes from frogs that came into my possession were given me by Dr. J. P. Hill, who was, at that time, on the staff of the Sydney University, but who, soon afterwards, went to London to take up the duties of the Jodrell Chair of Zoology at University College. In the follow-

ing two or three years, I added considerably to the number of these Trematodes mainly from two species of frogs, viz., *Hyla aurea* and *Limnodynastes peronii*, which are amongst the commonest and most easily catchable frogs in the neighbourhood of Sydney; and are, in consequence of this, the frogs most used in the biological laboratory here. These Trematodes included examples from the lungs (*Pneumoueces*), bladder (*Gorgodera*), duodenum (*Dolichosaccus*), small intestine (*Brachysaccus*), and rectum (*Diplodiscus*).

During the years 1910 and 1911, collecting frogs from various parts of New South Wales, I secured and examined for Entozoa the following species†:—

CYSTIGNATHIDÆ—*Micophyes fasciolatus*(2).

\**Limnodynastes peronii*(150),

\**L. dorsalis*(32).

\**L. tasmaniensis*(77).

*Crinia signifera*(12).

*Hyperolia marmorata*(12).

BUFONIDÆ - *Pseudophryne bibronii*(10).

HYLIDÆ - \**Hyla cærulea*(49).

*H. peronii*(12).

\**H. phyllochroa*(34).

*H. dentata*(6).

\**H. citropus*(2).

\**H. ewingii*(32).

\**H. aurea*(300).

\**H. lesneuri*(12).

\**H. freycineti*(26).

*Micophyes fasciolatus*.—The two specimens of this frog apparently contained no Entozoa of any kind.

*Limnodynastes peronii*.—At first, unfortunately, no record of the number of specimens examined was kept, but approximately about 150 specimens have been examined and the following

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† The number following the name of the frog gives the number of specimens examined, while the asterisk indicates that Trematodes were found.

Trematodes obtained— from the lungs, 2 (*Pneumonæces*); duodenum, 214 (*Dolichosaccus*); rectum, 10 (*Diplodiscus*); bladder, 23 (*Gorgodera*). In addition, nematodes were found, a number of times, in the lungs, intestine and rectum.

*Limnodynastes dorsalis*.—Out of the 32 specimens examined, trematodes were met with only once, one frog yielding two specimens of *Dolichosaccus*, which occurred in the anterior part of the small intestine. Nematodes, however, were fairly common, being found, a number of times, in the stomach, intestine and rectum.

*Limnodynastes tasmaniensis*.—In 77 specimens, trematodes were met with only once, the rectum of one frog containing three specimens of *Diplodiscus*. Nematodes occurred in the lungs, stomach and rectum.

*Crinia signifera*.—The 12 specimens examined contained no trematodes, but nematodes were found in the stomach and intestine.

*Hyperolia marmorata*.—Twelve specimens were examined, but neither trematodes nor nematodes were found, though in the intestine of two specimens, three cestodes were discovered, one in one, and two in the other.

*Pseudophryne bibronii*.—The ten specimens examined yielded no entozoa at all.

*Hyla cœrulea*.—The 49 specimens examined yielded in all 104 trematodes. In the duodenum of one frog were 4 (*Mesocelium*); in another, 92 of the same fluke; in another frog were 7 specimens of *Dolichosaccus* in the anterior part of the intestine, whilst in the rectum of two other frogs were three specimens of *Brachysaccus*. Nematodes were also found in the stomach, intestine and rectum; and cestodes in the rectum.

*Hyla peronii*.—Twelve specimens of this species yielded no trematodes, but nematodes were found in the lungs and rectum.

*Hyla phyllochroa*.—In the bladders of these frogs, of which 34 were examined, 15 specimens of *Polystomum* were found. Four was the greatest number in one specimen, two was the number most commonly met, and in several cases, one alone. Nematodes were found in the rectum of two specimens.

*Hyla dentata*.—Only six specimens of this rather rare frog were obtained, and no trematodes found, but nematodes were obtained from the intestine of one.

*Hyla citropus*.—Only two specimens of this comparatively rare frog were obtained; and from the duodenum of one, two flukes (*Mesocœlium oligoon*) were obtained.

*Hyla ewingii* is a fairly common frog near Sydney, and from 32 specimens, two trematodes were obtained, one (*M. megaloon*) from the intestine, while another specimen contained a single *Diplodiscus* in the rectum. Nematodes were also obtained from the intestine and rectum.

*Hyla aurea* is the commonest frog in the neighbourhood of Sydney, and over 300 specimens were examined for entozoa. From the lungs, 59 specimens of *Pneumonæces* were obtained; they occurred sometimes singly in one or each lung; in one case, as many as 10 were found in the two lungs. In the anterior part of the intestine of one frog, 35 specimens of *Dolichosaccus* were found; and one specimen of *Pleurogenes*; further back in the intestine, *Brachysaccus* was found fairly frequently, 205 specimens altogether being obtained. The rectum yielded 19 specimens of *Diplodiscus*, which occurred sometimes singly, sometimes in twos or threes. From the bladder, 75 specimens of *Goryodera* were got. Nematodes are very common in this frog, and large numbers were got from the lungs, intestine and rectum. Three cestodes were found in the intestine, and a large number in the muscles.

*Hyla lesueurii*.—Of 13 specimens of this frog, only one contained trematodes, and that a single specimen of *Polystomum* in the bladder. These frogs were collected at the Lodden River, in the same locality in which *Hyla phyllochroa* occurred. Nematodes were found in the rectum.

*Hyla freycineti*.—In the 26 specimens available, trematodes occurred twice, 7 specimens of *Pleurogenes* being found in the intestine of one frog, and 15 specimens of *Dolichosaccus* in the duodenum of another. Two cestodes were found in the duodenum of one specimen.

## SECTION iii.

*Methods employed.*—In looking for trematodes in frogs, after inspecting the buccal cavity (up to the present no representatives of the European *Halipegus* have been found by me, in Australian frogs), the ventral body-wall was slit from vent to chin, and the whole of the alimentary tract from pharynx to anus, including liver and lungs, removed to a shallow dish of normal saline solution. In the body-cavity of the frogs examined, so far, no trematodes have been found; in fact, a few nematodes represent the only helminths found in this situation. The whole length of the alimentary canal was then slit open, and its internal surface, lying submerged in salt solution, carefully brushed with a camel's hair brush. In most cases, the trematodes could be plainly seen before the brushing began; in only a few cases did the brushing discover worms whose presence was not obvious before. Finally, the liver, gall-bladder and lungs were looked into. Only in a few cases did I apply the method of shaking in a tube of salt solution, recommended by Looss(48, 53), namely, in the case of *Pneumonæces* from the lungs, and *Gorgodera* from the bladder. Without some such process, it is certainly not very easy to get pretty straight and extended specimens of these species, but in all the other species, as well as many times in those two just mentioned, quite satisfactory specimens for examination were obtained by methods given below. Worms intended for whole-mounts were placed on glass slides, each in a drop of salt solution and covered with a coverglass; and a number of these slides placed in a flat glass dish. Some step has to be taken to prevent the coverglass from being washed away when the fixing fluid is added, and I accomplished this by placing, on each coverglass, a small glass bottle, about 8 mm. wide by 20 mm. long. If the amount of fluid under the coverglass is sufficient, the weight of this bottle extends the worm very little indeed. By regulating the weight of the bottle for the larger and stronger worms by means of a little drop of mercury poured into the bottle, the worms will be found, after a short interval, to be in a state of extension, approaching very nearly that which they have when lying free in a state of rest, as regards their length; in most



cases, however, they are somewhat broader, especially those that have a round rather than a flat cross-section. The whole is now flooded with the fixing solution. I have obtained the best results by using boiling sublimate acetic, or sublimate acetic alcohol—a saturated solution of corrosive sublimate in water or 70% alcohol respectively, with 2% glacial acetic acid added. These solutions used cold, and Flemming's strong solution also give good results. In a few seconds, the little bottles are lifted off for a moment, when it may be seen, by the opacity of the tissues round the edges, that the fluid is already taking effect. In from half a minute to two or three minutes, the whole body has become opaque, and the bottle-weights may be removed, and the cover-glasses washed off by disturbing the fluid. The worms are now left in the fixing fluid for from half an hour to two or three hours, and then treated in the way usually employed for the after-treatment of tissues fixed in the fluid used, whichever it may have been.

Another method, which gave excellent results, for fixing the worms for whole-mounts, suggested to me by my chief, Professor Haswell, is this—a coverglass, with two little dabs of vaseline near the edges, is inverted over a worm lying in a drop of salt solution on a slide. By means of the dabs of vaseline, the pressure on the worm may be made as little or as great (up to a certain extent) as we please. In this way, the most delicate worms do not become squeezed too much, while the larger ones (of course no very large muscular flukes occur in frogs) are held firmly enough in the required position till they are fixed. But in this method, only cold fixing solutions, which do not act so quickly as the hot, can be used. I have not found the spines or processes of the cuticle injured by these reagents, as, for instance, Stafford has(88), in using glacial acetic acid.

In fixing the worms for sections, after washing in salt solution to remove slime, etc., the worms are allowed to come to rest in a little drop of salt solution in the bottom of a dish, and are then flooded with a comparatively large bulk of fixing fluid. The boiling sublimate acetic kills them practically instantaneously, and with the least amount of contraction; the Flemming also

kills them quickly, but they contract a little more. In both cases, subsequent investigation shows the tissues to be excellently fixed.

Sections were cut in three directions; in the case of flat worms, the horizontal longitudinal sections, that is, cut parallel to the dorsal or ventral surface, gave particularly pleasing pictures; for these sections, the worms were fixed under coverglasses, with just enough pressure to keep them fairly flat. Where the amount of material allowed, large numbers of each of the species examined (20-30 worms) were sectioned. The sections were cut in thicknesses varying from 5 to 10  $\mu$ .

For staining whole-mounts, hæmatoxylin gave, on the whole, the best results, though safranin, borax-carmin and paracarmine were very good. Safranin was specially useful for staining yolk-glands in those specimens where the yolk-glands are more or less hidden from view by other organs, for the safranin still stained these glands brilliantly when it was almost completely washed out of all the rest of the tissues. For staining the sections, Ehrlich's hæmatoxylin followed by eosin gave uniformly good stains; paracarmine was also very useful. Neither of these stains, however, was any good for Flemming-fixed material, and for this iron-alum hæmatoxylin was used. Very good results were obtained by using, on Flemming-fixed material, a modification of a process suggested by Dr. Arnold of the Institute of Tropical Medicine, Liverpool. The stains used in this method are safranin, methylene blue and orange G. From 50% alcohol, the sections are brought into an iodine mordant (40 c.c. abs. alc., 2 gm. iodine crystals, 60 c.c. distilled water, 5 gm. potassium iodide) for 5 minutes, washed in tapwater for 30 seconds, and placed in safranin (the aniline-water formula) for 24 hours; wash in tapwater and put into methylene blue for 30-45 minutes; wash in tapwater one minute, run through 30, 50, 70, 90 per cents. alcohol, 30 seconds in each, absolute alcohol 2 or 3 minutes; orange G in clove oil 5-10 minutes; xylol; and mount in Canada balsam.

By this process, the methylene blue stains the chromatin blue, the safranin stains the egg-shells and the parts of the yolk-glands that furnish the material for the egg-shells (Goldschmidt, 26) a

brilliant red, while the muscle and connective tissues, cuticle and spines are stained light to dark brown by the orange G. The chief drawback is that these stains are not nearly so permanent as the hæmatoxylin.

For the excretory system, besides the living worms, in which it is best studied, unstained specimens, cleared in xylol-phenol and mounted in Canada balsam, were most satisfactory.

In addition to working on fixed and stained material, I have also, as Looss(48,53), Lühe(10,60) and others recommend, studied the worms alive, mounted in salt solution. Most of these worms will keep alive for 48 hours or longer in salt solution, and will even live mounted on a slide and slightly compressed by the cover-glass, for a number of hours. The slight pressure makes the worms sufficiently transparent for one to be able to make out practically all the details of their internal structure. It was while working in this way, that I was able to follow out the process of egg-formation in *Dolichosaccus*, as well as to see the Laurer's canal in action, and to observe the early stages in the development of this worm. A special section will be devoted to the explanation of these processes.

#### SECTION IV.

*Systematic.*—In this section, the trematodes found are described and referred to their places in the system. For each species, a brief diagnosis is given, in which the special specific characters of the worm are emphasised by being printed in italics; the diagnosis is followed by a fuller description of the anatomy. I should consider this fuller description necessary, even if the worm described should show no striking anatomical differences from its representatives in other zoogeographical regions, because, without such a description, it is often very difficult for a person knowing the worm by the description only, to rightly judge what its relationships may be. Where several species of the same genus occur here, however, (*e.g.*, *Pleurogenes*, etc.) there is no need to describe the anatomy of more than one of these fully. The special characters being picked out in the diagnosis, it may be taken that the rest of the anatomy closely corresponds to that of the species more fully described.



The anatomical description is followed by general remarks, and a discussion of the relationships of the worm described. For the new genera, descriptions with the more special characters emphasised by italics have been given, as this procedure appears to me to be more satisfactory than that of designating a genus by merely naming its type-species. The worms here described are:—

Order HETEROCOTYLEA Mont.

Family POLYSTOMIDÆ Taschbg.

Subfamily **Polystominæ** van Ben.

Genus POLYSTOMUM.

Species—*bulliense*.

Order MALACOCOTYLEA Mont.

Family PARAMPHISTOMIDÆ Fisch.

Subfamily **Cladorchinæ** Fisch.

Genus DIPLODISCUS Dies.

Species—*megalochrus*, *microchrus*.

Family FASCIOLIDÆ Rail.

Subfamily **Plagiorchinæ** Lühe (**Lepodermatinæ** Looss).

Genus DOLICHOSACCUS, gen.nov.

Species—*trypherus*, *ischyrus*, *diamesus*.

Genus BRACHYSACCUS, gen.nov.

Species—*anartius*, *symmetrus*.

Subfamily **Haplometrinxæ** Pratt.

Genus PNEUMONÆCES LOOSS.

Species—*australis*.

Family GORGODERIDÆ LOOSS.

Subfamily **Gorgoderinxæ** Looss.

Genus GORGODERA LOOSS.

Species—*australiensis*.

Family BRACHYCÆLIIDÆ.

Subfamily **Brachycæliinxæ** Looss,

Genus MESOCÆLIUM Odhn.

Species—*mesembrinum*, *oligoon*, *megaloon*.

Subfamily **Pleurogenitinxæ** Looss.

Genus PLEUROGENES LOOSS.

Species—*freycineti*, *solus*.

## Order HETEROCOTYLEA.

## Family POLYSTOMIDÆ,

Subfamily **Polystominæ.**

## POLYSTOMUM BULLIENSE, sp.n.

(Figures 1, 2, 18-28.)

*Diagnosis.* — Flattened oval worms, 3 mm. long by 1 mm. broad; with a somewhat circular cotylophore marked off into six rounded lobes, each bearing a circular sucker. Larval hooklets on the disc and suckers; and two large falciform hooks bearing a bilobed crest, present. Well-marked *vaginal swellings*. Œsophagus wanting; intestinal limbs *reaching into the caudal disc, without transverse anastomosing branches*. Testis a flat plate of follicles in the middle of the ventral surface of the body; a single vas deferens; a crownlet of *eight* penis-spines. Ovary a large oval body obliquely placed. Genito-intestinal canal present. Yolk-glands extending over the *whole dorsal surface from near the genital opening, right back into the caudal disc*. Uterus a straight wide tube, running from the posterior end to the genital opening. Eggs thick-shelled, bright yellow, large, 0.206 mm × 0.095 mm.

In the bladder of *Hyla phyllochroa* and *H. lesueurii*.

Type-specimen in the Australian Museum, No. W.346.

Sixteen specimens of these worms, which may be looked upon as the Australian representatives of *P. integerrimum*, were obtained, one from *Hyla lesueurii* and the rest from *H. phyllochroa*. These frogs were obtained from a tributary of the Lodden River, near Bulli, N.S.W., in October and November. The worms occurred in the urinary bladder, and sometimes only a single specimen was found; usually there were two, and once as many as four were found in one bladder.

The worm is a narrow oval in shape, with a wider, six-lobed cotylophore at the posterior end, bearing six circular suckers, near the margin, one on each rounded lobe. The body is fairly flat, transverse sections being a flattened oval in shape, less flattened towards the anterior and more flattened towards the

posterior end, till in the region of the cotylophore, both ventral and dorsal surfaces are quite flat. The body is 2.93 mm. long, and 1.007 mm. broad in front of the disc; the latter is 1.4 mm. broad. The suckers on the disc are fairly circular in outline, 0.268 mm. in diameter, and are arranged round its edge, more or less in a circle. The radial muscles of these suckers are very conspicuously developed. In the centre of each sucker, the muscular tissue is perforated by a plug of connective tissue (Fig. 18) which runs through to the surface, and lying in this tissue, is a strong bundle of muscle-fibres, placed at right angles to the surface, the action of which is to draw back the bottom of the sucker in the process of attachment. Four larval hooklets are present in a row on the ventral surface near the posterior edge of the disc or cotylophore. The hook resembles a button-hook; the shaft is bifurcated (Fig. 1b). I have been able to find no trace, either in the living worms or in the fixed material, of the larval hooklets which *P. integerrimum* and other species of this genus bear near the anterior edge of the disc. There is a small anchor-shaped hook in the centre of each sucker. All these hooklets either disappear as the animal increases in age, or very readily become detached. In only one out of sixteen specimens have the whole four posterior hooklets been present; and in only two others were any hooklets at all to be seen. In all the other specimens, no hooklets could be made out. But the pair of large chitinous hooks, lying between the two most posterior suckers on the ventral surface of the disc, is present. These hooks are flat plates dorso-ventrally; but, in surface-view, they have a wide posterior border, which is generally split up by two or three incisions, and the general shape is that of a reaping hook with the point bent sharply back (Fig. 1a). Just at the bend, the hook bears a bifid, crest-like process. These hooks are 0.15 mm. broad at the base, and have a length, measured in a straight line from the base to the height of the bend, of 0.29 mm., while the recurved, claw-like terminal portion measures 0.05 mm.

At the sides of the body, near the junction of the first and second body-fourths, the vaginal openings are situated on two well-marked papilla-like elevations.

The mouth-opening is terminal and anterior, and leads into a fairly spacious buccal cavity, which is really an incipient oral sucker, being surrounded by distinct layers of longitudinal, circular, and radial muscle-fibres, so that in sections it presents exactly the appearance of a weakly-developed sucker(Fig.19). The pharynx, in the form of a muscular, oval bulb, opens directly into this oral sucker, but a diverticulum from the buccal cavity runs backwards, ventral to the pharynx, and for a distance equal to its length, forming a median, unpaired buccal pocket(Fig.20). A median and two lateral groups of large gland-cells are situated behind and to the sides of the pharynx. The ducts of these so-called "salivary glands" are closely applied to the inner surfaces of the wall of the pharynx for a very short distance, when they open into its cavity. There is practically no œsophagus, the pharynx opening directly into the bifurcated intestine. The intestinal limbs are pretty straight when empty; but mostly they are full of dark brown or black granules. When this is the case, the walls are thinner, and their surface somewhat sacculated. They run backwards pretty near the lateral edges of the body, and end in a position dorsal to the cotylophore, and just in front of the posterior pair of suckers(Fig.26). When the uterus is distended, they diverge at the posterior extremities. The transverse anastomosing branches characteristic of *P. integerrimum* are quite absent.

The excretory vesicles(Fig.21) lie near the vaginal papillæ close to the dorsal surface, on which each opens by a minute pore. The main vessels proceed backwards to a position in the posterior end of the body behind the intestinal limbs and uterus, where they join one another. In this region, a pair of branches is given off, which run forwards to near the anterior end, giving off a few smaller branches and capillaries that end in flame-cells.

The most conspicuous part of the genital system is the ovary, a large oval body lying somewhat obliquely placed on the right side, just behind the vaginal papillæ. The testes are in the form of a large number of oval or rounded follicles which, lying closely packed together, begin just behind the ovary, and extend in a single layer on the ventral surface back to a position halfway

between the ovary and the anterior edge of the cotylophore, whilst laterally they do not quite reach the sides of the body (Figs.1 and 24). The single vas deferens springs from the middle of the anterior surface of the testes, and runs forwards and dorsally so that it passes the ovary on its dorsal side, and pursues a pretty straight course to the level of the genital opening, when it bends towards the ventral surface and terminates at the end of the penis. For about a third of its course, near the ovary, it is a pretty wide tube, and is filled with sperms, so that this part may be looked upon as a vesicula seminalis; towards the end of its course, its walls become thickened to form the ejaculatory duct. Two laterally placed, small groups of gland-cells, lying near the beginning of the duct, represent the prostate. The end of the penis is armed by a crown of eight curved chitinous spines. The male duct opens into a distinct genital chamber, which opens on the ventral surface in the mesial plane, about halfway between the ovary and the anterior end.

The ovary is a large oval body, obliquely placed, generally on the right side, with its narrower end anterior and ventral. This end is alone the germinal area, and here the ova are very small, while they gradually increase in size to the other end, where the large, ripe ova are found (Figs.1 and 22). The oviduct coming off from this end, runs at first ventrally, and soon widens to form the ootype, which is surrounded by the cells of the shell-gland; the main unpaired duct of the yolk-glands and the genito-intestinal canal open into the oviduct, close together, just in front of the ootype (Fig.2). From the ootype, the oviduct runs forwards to a point in front of the ovary, when it bends sharply backwards and runs in a straight course close to the ventral surface, almost to the level of the cotylophore, where it opens into the wide uterus. The uterus is a wide, elongated sac, extending back to the extreme posterior end of the body, in its posterior part, when it is distended with eggs, occupying almost the whole width of the body. It runs forward in a dorsal position (Figs.24, 25), along the middle line of the body, in a straight course to near its termination, when it runs ventrally and opens into the genital chamber, ventral to the male opening.



The lateral, vaginal swellings are formed by a large number of papillæ, perforated by fine canals (Fig. 28), which, after a very short course, open into a fairly wide sperm-reservoir (Fig. 23), situated, one on each side, just under the swellings. From these reservoirs, a wide vaginal tube on each side runs backwards and inwards, to open into the anterior lateral yolk-duct. On one side only, in the specimens sectioned, was the vaginal tube filled with sperms; that on the other side was empty.

The yolk-glands are very extensively developed, stretching from a level just behind the pharynx, right back into the caudal disc. They consist of a large number of oval, closely-packed follicles, and stretch across the whole dorsal surface of the body, except in those cases where the uterus is loaded with eggs. When this is the case, the uterus occupies the middle area of the dorsal surface, and the yolk-glands are pushed to the sides, for a part, at least, of their course (Figs. 24, 25). The anterior and posterior lateral yolk-ducts, on each side, join at a level just behind the ovary, to form two transverse ducts which run inwards and unite to form a large yolk-reservoir that lies behind the ovary. A single duct from this reservoir opens into the oviduct near the ootype. The genito-intestinal canal enters the oviduct close to the opening of the yolk-duct, and runs, first posteriorly, then, bending sharply, it proceeds transversely across the body, in a gently curved course, to open into the intestine on the same side, *i.e.*, the right (Fig. 27).

The wall of the canal passes obliquely through the wall of the intestine, and projects a little into the lumen (Fig. 2), so that a kind of valve is formed, which would prevent substances passing from the intestine into the canal.

The eggs are oval, bright yellow in colour, large and thick-shelled, 0.206 mm. long, by 0.095 mm. broad; not so large as the size (0.22-0.24 × 0.16 mm.) given by Braun (4, p. 499) for the eggs of *P. integerrimum*.

Literature, 2, 4, 17, 27, 28, 30, 35, 75, 79, 93, 104, 105.

## Family PARAMPHISTOMIDÆ Fischdr.

## Subfamily Cladorchinæ Fischdr.(?).

## DIPLODISCUS MEGALOCHRUS, \* sp.n.

(Figures 29, 31-42.)

*Diagnosis.*—Subclavate worms, 3-4 mm. long, by 1.25 to 1.5 mm. broad. Integument smooth. Suckers comparatively large; *ratio of anterior sucker to posterior*, 3:10. Diverticula of the oral sucker well developed; œsophagus long, pharynx well-developed, at the posterior end of the œsophagus. Intestinal limbs moderately wide and short. *Excretory pore at the extreme posterior end of the body.* Genital opening *behind the intestinal fork.* The single testis large and generally somewhat cubical; a large vesicula seminalis arranged in a number of coils. Copulatory organs present. Ovary *oval*, behind the testis; shell-gland about as large as the ovary. Vitelline glands of large follicles, partly in a single, and partly in a double row along the sides of the body, outside the intestinal limbs. Coils of the uterus occupying a great part of the body-space, from the posterior end to the genital opening. Eggs with thin, hyaline shell,  $0.132 \times 0.066$  mm.

In the rectum of *Hyla aurea* and *Limnodynastes peronii*.

Type-specimen in the Australian Museum, No. W.332.

This species of *Diplodiscus* occurs in the rectum of *Hyla aurea* and *Limnodynastes peronii*. I have obtained 19 specimens from the former, and 10 from the latter, 15 in all being sectioned.

The worm is somewhat club-shaped, wider a little in front of the posterior sucker, very bluntly rounded behind: in front tapering to a much smaller diameter. Except near the anterior end, where it is fairly circular, the cross-section of the body is elliptical, but somewhat flattened dorso-ventrally. The average length is 3.72 mm., breadth 1.37 mm.

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\* μέγας, large; ὄχρος, yolk, referring to the large yolk-follicles.

The integument is perfectly smooth, without any trace of spines or tubercles. The opening of the oral sucker is terminal; that of the posterior sucker subterminal or postero-ventral. The average diameter of the oral sucker is 0.313 mm., of the posterior 1.085 mm.; the ratio of oral to posterior 1:3.46 or about 3:10.

The oral sucker is a thick, muscular subglobular structure, deeply embedded in the body of the worm, with a pair of diverticula, the so-called "pharyngeal pockets," given off laterally from its base. These diverticula are about as deep (0.33 mm.) as the main body of the sucker, and are joined together in the middle line for the most part of their length, only being completely separated from one another near their terminations (Figs. 37, 38). The structure of the walls of these pockets is exactly similar to that of the oral sucker, and the arrangement of its muscle-fibres, radial, circular, longitudinal and oblique, together with the embedded cells, agrees very closely with the detailed description given by Looss (55) for the oral sucker of *Amphistomum spinulosum*. At the bottom of the sucker, lies the opening of the œsophagus, running into it from a ventral position, and lying very close to the openings of the diverticular (Fig. 38). The œsophagus is a long tube with fairly thin walls, running almost straight back to the forking of the intestinal limbs, with a length of about 0.52 mm. At its posterior end, the muscular wall of this tube becomes considerably thickened so as to form a pharynx (0.12 mm. long), which opens into the intestine. Fiscoeder (19), Daday (16), and others have sought to make the structure, which I have called the oral sucker, homologous with the pharynx of other trematodes. I cannot agree with this, but concur with Looss' contention (55, p. 440) that here we have to do with a real oral sucker. Leaving out of account for the moment the *Amphistomes*, one finds, in most of the *Malacocotylea*, an oral sucker, out of the base of which, that part of the alimentary tract lying between it and the intestine, and called the œsophagus, opens. On the course of the œsophagus some

where, is a thick-walled muscular bulb (wanting, however, in the *Gorgoderinae*, and a few others), which is almost universally called the pharynx. The exact position of this pharynx is subject to a good deal of variation; in most *Fasciolidae*, following almost immediately upon the oral sucker; in many others, e.g., the *Lepodermatinae*, *Dolichosaccus*, *Brachysaccus*, the pharynx is placed further back, a thin-walled section, the prepharynx, being interposed between the pharynx and the oral sucker (Fig. 43). In others, again, the pharynx is placed much further back as, for instance, in *Microscaphidium* (55, p. 691), where it opens directly into the intestine. Just in this position does a well-marked pharynx lie in *Diplodiscus*, as the photographs 36, and 34 and 35, of transverse and horizontal longitudinal sections respectively, show. The wide, short, intestinal limbs are circular in transverse section, and reach just past the middle of the body. The posterior sucker is, in life, ventrally directed, but in most preserved specimens it faces postero-ventrally. In form, it presents a quoit-like rim and a deep central cavity, in the centre of which, concentric with the outer rim, is the second quoit-like ring, the meaning of which has given rise to so much discussion (Braun 4, p. 693). Its centre is often deeply depressed from the exterior, while a cylindrical plug of parenchyma cells projects towards its centre from within, the two opposing depressions making the thickness of the sucker, in this region, much less than elsewhere (Figs. 32, 33). Through this depression, there is no opening of the excretory system nor of any other system of ducts or vessels. The muscular ring is entirely an accessory part of the sucker, and, no doubt, is of considerable assistance in perfecting the clinging action of the whole sucker. When the sucker is being fastened on to the wall of the frog's bladder or any other surface, this central plug would assist in making the expulsion of the air (or gas, or whatever its contents may be) from the cavity of the sucker much more complete, in just the same way as the columnæ carneæ and muscoli pectinati of the mammalian heart assist in expelling the last drop

of blood from the contracting ventricle. Then the muscle-fibres of this part of the sucker are arranged in such a way that this central plug can be drawn far back from the surface to which the worm is attached, so as to give the sucker a very powerful clinging action.

The cells of the body-parenchyma are very large and thin-walled, and more or less filled with finely granular contents. Between these cells are two longitudinal vessel-like spaces, which Looss (55, p. 432) has described, in *A. spinulosum*, as lymphatics. They run from the oral to the posterior sucker, and enter into intimate relation with the suckers.

The excretory vesicle lies in the body directly behind the posterior sucker. From its anterior end, a pair of large vessels come off, running first pretty straight outwards to the sides; then they turn forwards, and run in a somewhat wavy course up to the level of the oral sucker diverticula; crossing the intestine on the ventral side, they then bend sharply back, and run as much finer tubes to the posterior end of the body, giving off, at various points, capillaries that end in excretory cells. Some branches of the excretory system enter the posterior sucker, but the arrangement of these has not been closely followed out. At its posterior end, the vesicle gradually narrows into a moderately fine duct with muscular tissue in its walls, and this duct opens on the exterior by an exceedingly minute pore, less than 0.005 mm. in diameter, in every one of my sectioned specimens, more than a dozen in number. This pore is situated right at the posterior end of the body, just near the posterior rim of the sucker.

The genital opening (Fig. 39) lies in the middle line on the ventral surface, just behind the intestinal fork. It leads into a small genital chamber, into which the male and female ducts open.

There is a single testis, in the form of a large, somewhat cubical or rectangular body, laterally placed near the intestine and internal to it. This testis is inconspicuously marked off into two lobes by a groove running along its ventral and

anterior surfaces. The two vasa deferentia soon join to form a wide thin-walled tube, the vesicula seminalis, which after a course of two or three turns, transversely placed, enters a muscular sac, which may perhaps be called the cirrus-sac, though not entirely in agreement with that sac in other trematodes, for towards its outer end, the female duct also enters it from the ventral side. Several coils of the vesicula seminalis occupy the posterior part of this sac, and in front gradually narrow down to the much thicker-walled ejaculatory duct. The vagina enters the sac near its distal end, occupying a position ventral to the male duct. The rest of the cavity of the rather wide sac is filled up by parenchyma-cells and some prostate-cells.

The ovary is an oval body, also laterally placed, lying some distance behind the testis, just behind the level where the intestinal limbs end. The oviduct is given off from the dorsal side, and after a short course, enters the mass of shell-glands (Fig. 42), lying over the ovary, just under the dorsal surface of the body. Just in front of the shell-gland, the Laurer's canal takes its origin, and runs forward for a distance of 0.15 mm., to open on the dorsal surface near the middle line. From the shell-glands, the uterus runs down to the posterior end of the body and back in several wide coils; behind the testis, several coils occupy a rather dorsal position, but in front of that body, they are more ventrally placed; near the cirrus-sac, the lumen becomes considerably narrowed, and the walls thicker to form the vagina. The first part of the uterus, near the shell-gland, is filled with spermatozoa, and may be looked upon as the receptaculum seminis uterinum. The yolk-gland is composed of large (0.186 × 0.123 mm.) oval follicles, which run in a single or double row from a position behind the diverticula of the oral sucker, to a level just behind the ovary; in this part of their course, they are laterally placed, lying for the most part between the intestinal limbs and the lateral body-wall. Behind the intestinal limbs, on each side, they turn sharply inwards and stretch

across to meet in the middle of the body, forming a dorsally-placed row posterior to the ovary.

The eggs are large (0.132 mm. long, by 0.066 mm. broad), with a thin, hyaline shell: many of them contain ciliated embryos. These measurements are smaller than those given by Looss (46) for the mature eggs of *D. subclavatus*, viz., 0.14 × 0.07 mm. Dujardin's figures are 0.13 mm. long, while Stetti states the length as 0.19 mm. (4, p. 766): perhaps Stetti had to do with an independent species.

DIPLODISCUS MICROCHRUS, sp.n.

(Figure 30.)

*Diagnosis.*—Small, subclavate worms, about 1.2 mm. long, by 0.85 mm. wide. Suckers large; *ratio of anterior to posterior*, 3:8. Diverticula of the oral sucker well-developed; œsophagus long; pharynx well-developed, and placed at the posterior end of the œsophagus; intestinal limbs very wide and short. Excretory pore at the extreme posterior end of the body.

The single testis rectangular, laterally placed, near the middle of the body; a large vesicula seminalis; copulatory organs present. Ovary *pear-shaped*, behind the testis. Vitelline glands *comparatively small*, in a double row along the sides of the body and across the body behind the ends of the intestinal limbs. Coils of the uterus as in the last species. Eggs thin-shelled (0.125 × 0.067).

In the rectum of *Hyla ewingii* and *Limnodynastes tasmaniensis*.

Type-specimen in the Australian Museum, No. W.333.

Of this little species, I have obtained three specimens from *Limnodynastes tasmaniensis*, and one from *Hyla ewingii*. It differs from the preceding species mainly in the smaller size of the body, and the comparatively (to the size of the

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\* μικρος, small; ὄχρος, yolk.

body) larger suckers, while the ratio of the diameter of the suckers is different in the two species. In this species, too, the ovary is pear-shaped, and the follicles of the yolk-glands ( $0.09 \times 0.07$ ) are much smaller than in *D. megalochrus*. The eggs, too, differ in shape from those of the last-named species, being shorter and broader.

These two species are pretty closely related in their structure to their European representative, *D. Subclavatus* R., and somewhat less closely to the American *D. temperatus* Staff. They differ from the former in the more marked development of the oral diverticula, in the shorter intestinal limbs, in the position of the ovary in regard to the testis (according to Looss' figure in 46), and in the more posterior position of the excretory pore, as well as in the details of the arrangement of the collecting tubes of the excretory system. They differ from the American species in their much shorter intestinal limbs, in the testis being single, while there are two in *D. temperatus*; in the lateral position of the ovary rather than median, as in *D. temperatus* (91, p.689), and in the yolk-glands extending much further anteriorly.

These two Australian species, perhaps, ought to be placed in a separate genus, but I do not feel myself sufficiently familiar with the anatomy of related worms of this group (the published descriptions in most cases not going far enough) to make the attempt at present.

Literature referred to—4, 15, 16, 17, 19, 46, 55, 61, 63, 91.

#### Genus *DOLICHOSACCUS*, gen.nov.

Subfamily *Plagiorchinæ* Luhe, (*Lepodermatinæ* Looss).

(Figures 4-8, 43-50.)

*Diagnosis*.—Small, elongated worms of somewhat flattened, oval cross-section, about 3 mm. long, a little narrowed in front and behind, and rounded off at the ends. Integument spiny; suckers well-developed, not far apart. Pre-pharynx,

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\* *δολιχος*, long; *σακκος*, a sac, referring to the cirrus-sac.



pharynx, and œsophagus present; intestinal limbs reaching to the posterior end of the body. Excretory vesicle Y-like, *with a very long stem and short arms*. Genital opening near middle line, just behind fork of intestine, and in front of ventral sucker. Large, rounded testes in the posterior half of the body, *one behind the other in the middle line*; large rounded ovary, some distance in front of the testes. Copulatory organs present; *S-shaped cirrus-sac very long*, and well developed; vesicula seminalis constricted in the middle; Laurer's canal strongly developed, very long and convoluted; receptaculum seminis doubtful. Vitelline glands extensively developed, laterally placed in the anterior half of the body, but in the posterior half spreading all round the body, close under the surface. Uterus comparatively short, except for a very short part of the proximal portion, *lying in front of the ovary*.

In the *intestine of frogs*.

Type: *D. trypherus* sp.n.

#### DOLICHOSACCUS TRYPHERUS, sp.n.

(Figures 4, 5, 6, 43, 44, 45, 47.)

*Diagnosis*.—Small, elongated, *delicate* worms, about 3 mm. long, becoming gradually narrower towards the ends, and rounded off in front and behind. Integument *delicate*, spiny; suckers not far apart; oral sucker larger than ventral, ratio of oral to ventral 7:5. Excretory vesicle as in the genus. Genital pore just behind the intestinal fork. *Testes larger than the ovary*, one close behind the other, in the middle of the posterior half of the body. Ovary *some distance behind the ventral sucker, to one side of the middle line*. Copulatory organs and Laurer's canal as in the genus. Vitelline glands of *comparatively small follicles*, in front of the ovary not extending inwards beyond the intestinal limbs, and *anteriorly*

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\* τριψερος, delicate.

not reaching in front of the intestinal fork; behind the ovary gradually spreading under the whole surface of the body. Uterine loops mainly in front of the ovary. Eggs, light in colour,  $0.037 \times 0.022$  mm.

Hosts: *Limnodynastes peronii* and *Hyla aurea*, in the duodenum.

Type-specimen in the Australian Museum, No. W.334.

These worms are fairly common in the duodenum of *Limnodynastes peronii*, and much less common in *Hyla aurea*. At first, no record was kept of the frequency of occurrence, but in the last 47 of *L. peronii* examined, this fluke occurred nine times with a yield of 117 specimens. Altogether, I have collected 214 specimens of this species from 150-200 *Limnodynastes*; and 35 from *Hyla aurea*, in which I have found it only four times out of several hundred specimens examined. The greatest number found in one frog was 25, the lowest 4. They are very active and extensible, and are capable of stretching out to twice the length of the animal when in a state of rest. Thirty-five were cut into sections, and most of the rest mounted as whole-mounts. I cut so large a number, in order to have plenty of material for study in regard to the egg-formation and the early development, to supplement the observations made on the living worms.

These worms are of elongated, delicate form, oval in cross-section, but a little flattened dorso-ventrally, somewhat narrowed anteriorly and posteriorly, and rounded off at the ends; 2.98 mm. long, by 0.91 mm. broad. Integument densely covered by small backwardly directed spines, which have become much less dense at the middle of the body, and completely disappear before the end of the body is reached. Oral sucker subterminal, 0.243 mm. in diameter; ventral sucker placed at the beginning of the second quarter of the body-length, spherical, 0.169 mm. in diameter; ratio of oral to ventral sucker, 7:5. Opening of oral sucker on ventral surface; excretory pore terminal; genital pore in the middle line on the ventral surface, just behind the intestinal fork.

*Alimentary canal.*—The oral sucker opens behind into a pre-pharynx (Fig.43), nearly equal in length to the pharynx, which is a subglobular thick-walled structure, 0.140 mm. wide. There is a moderately long œsophagus, about equal in length to the pharynx and prepharynx taken together (Fig.43); the intestinal limbs run back to the posterior end of the body. The excretory vesicle (Fig.6) is Y-shaped, with a very long stem that divides into two short limbs just behind the ootype and related parts. From these limbs, a pair of moderately fine, anterior, collecting ducts run forwards in a lateral position, as far as the base of the oral sucker. About the level of the ventral sucker, or just behind it, a pair of posterior collecting ducts, much finer than the anterior ducts, arise from these, and run back to the posterior end of the body. Taking their origin from these collecting tubes, a large number of capillary tubes form a loose network all over the surface of the body (Fig.6); short branches, arising from the network at intervals, end in excretory cells. The number seems to vary a little in different individuals, but there are about 160 of these cells.

The testes lie in the middle line, one behind the other, in the middle of the posterior half of the body. They are large, compact, spherical bodies, nearly as large as the oral sucker, 0.214 mm. in diameter. The vasa deferentia run forwards, and do not join till they reach the base of the vesicula seminalis, which lies partly behind, and partly alongside the ventral sucker. The cirrus-sac is a very elongated, S-shaped structure, 0.611 mm. long (not following the bends, but measured in a straight line from its posterior to its anterior extremity), beginning behind, and to the right of the ventral sucker. In a graceful curve, it bends forwards and inwards to a position in front of the ventral sucker, and to the left of the middle line, whence, by a sharper bend, it runs forwards and to the right, to reach the genital pore, which lies just behind the intestinal fork and a little to the right of the middle line (Fig.47).

The posterior third of the cirrus-sac is occupied by the vesicula seminalis, which is constricted in the middle, affording, in its form, a very close resemblance to the swimming bladder of cyprinoid fishes (Fig.4), a resemblance mentioned by Looss for the vesicula seminalis of *Glossidium* (50). Anterior to this, is a well-developed pars prostatica and a strong protrusible penis, traversed by a very muscular-walled ejaculatory duct. The ovary is a large, rounded or somewhat oval body, a little smaller than the testes, 0.197 mm. in diameter, laterally placed on the right side just in front of the middle of the body, some distance behind the ventral sucker. The oviduct leaves the ovary on its inner posterior aspect, and runs transversely close behind the ovary for a distance of about 0.15 mm., when it opens by a comparatively wide opening into a sac-like structure, which I must call the fertilization-space, though, in fixed and stained whole-mounts, one would naturally call it the receptaculum seminis. A study of the living worms shows that the ova are fertilized in this space, just near its wide opening into the oviduct. The sac itself is of comparatively considerable proportions, measuring 0.121 x 0.101 mm. If this sac does not represent the receptaculum seminis, then no such structure is present in the species of this genus. Laurer's canal, which opens into the oviduct just beyond the fertilization-space, is especially strongly developed. It is a thick-walled, elongated tube, lying in the dorsal region of the body, and pursuing a tortuous, convoluted course backwards, as far as the level of the anterior testis, where it opens on the posterior surface (Figs.5,44). Just beyond its junction with Laurer's canal, the oviduct expands to form the ootype, which is surrounded by the cells of the shell-gland, a not very strongly developed structure. Into the ootype, opens the short duct from the somewhat conspicuous yolk-reservoir. Leaving the ootype, the female duct soon turns forward as the uterus, and, after crossing the body in several narrow, transverse coils, which lie in the region in front of the ovary, it runs forward to the genital opening.

The yolk-glands are extensively developed, and consist of oval follicles,  $0.058 \times 0.039$  mm. Anteriorly, they do not extend beyond the fork of the intestine, and, in front of the ovary, are confined to the sides of the body, not spreading inwards beyond the intestinal limbs. They are very usually unequally developed on the two sides, in the majority of the specimens, not extending forwards on the left side as far as the ventral sucker, while they reach a point just in front of it on the right side (Fig.45). Behind the ovary, they gradually spread inwards, lying close to the surface of the body till, in the region of the testes, or in some cases, just behind these, they form a thin but complete, more or less superficial layer surrounding the body (Fig.45). The rather thin-shelled eggs are broadly elliptical in shape, yellow to light brown in colour, comparatively few in number, and fairly large ( $0.037 \times 0.022$  mm.).

DOLICHOSACCUS ISCHYRUS\*, sp.n.

(Figures 7, 46, 48, 49, 50, 50a).

*Diagnosis*.—Small, elongated, fairly robust worms, about 3 mm. long, not much narrower at the rounded-off ends. *Integument thick*, spiny: suckers fairly close together; ratio of oral to ventral, 4:3. *Excretory vesicle very wide and long*. Genital pore just behind the intestinal fork. *Testes smaller than the ovary*, one close behind the other in the middle line, at the middle of the posterior half of the body. Ovary large, rounded or oval, *in the middle line, nearer the ventral sucker than in D. trypherus*. Copulatory organs and Laurer's canal as in the genus. Vitelline glands of *moderately large follicles, closely packed together*, extending anteriorly *right up to the oral sucker*, in front of the ovary laterally placed, but in the posterior part of the body spreading under the whole surface.

\* ἰσχυρός, robust.

Uterine loops mainly in front of the ovary. Eggs 0.042 mm. long.

Hosts: *Limnodynastes dorsalis* and *Hyla caerulea*, in the intestine.

Type-specimens in the Australian Museum, No. W.335.

Seven of these worms were found in the intestine of *Hyla caerulea*, and two in *Limnodynastes dorsalis*. They are less pointed at the ends than *D. trypherus*, and of stronger, more robust form, with a much thicker cuticle. The average length is 4.5 mm., and breadth 1.1 mm. The oral sucker varies from 0.245 to 0.318 mm. in diameter, while the ventral ranges from 0.179 to 0.245, the ratio between the two being 4:3. The excretory vesicle is very voluminous (Figs. 49, 50), being much wider than in *D. trypherus*. It differs from that species, too, in having the ovary (0.318 × 0.236 mm.) larger than the testes (0.27 × 0.228 mm.), and placed in the middle line. The testes are closer together. The yolk-glands consist of larger follicles (0.083 × 0.05 mm.), which are more closely packed than in the last species, and extend further forwards, reaching the base of the oral sucker.

DOLICHOSACCUS DIAMESUS,\* sp.n.

(Figure 8).

*Diagnosis*.—Small, elongated, fairly delicate worms, becoming narrower towards the extremities. Integument fairly thick, spiny; ratio of oral to ventral sucker, 4:3. Excretory vesicle *very wide and long*. Genital pore just behind the intestinal fork. Testes smaller than the ovary, in the middle line, in the middle of the posterior half of the body, with their *ends obliquely over-lapping*. Ovary close behind the ventral sucker and laterally placed. Copulatory organs and Laurer's canal as in the genus. Vitelline glands of compara-

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\* *διαμεσος*, intermediate.

tively small follicles, *extending anteriorly as far as the intestinal fork, and inwards beyond the intestinal limbs*, both in front of, and behind the ovary. Uterine loops as in the other two species. Eggs,  $0.036 \times 0.021$  mm.

Host: *Hyla freycineti*, in the stomach.

Type-specimen in the Australian Museum, No. W.336.

Fifteen specimens of this worm were found in the stomach of *Hyla freycineti*. In the robustness of its form, it stands intermediate between *D. trypherus* and *D. ischyurus*. The average length is 2.2 mm., breadth 0.65 mm.; oral sucker 0.189, ventral 0.145 mm. The relation in size of the suckers is about the same as in the last-named species, with which it agrees also in possessing a very wide and voluminous excretory vesicle, as well as in having the ovary ( $0.261 \times 0.212$  mm.) larger than the testes ( $0.245 \times 0.179$  mm.). It differs from this species, however, in the lateral position of the ovary, in the much smaller form of the yolk-gland follicles, and in the extension of these glands. On the whole, it appears to be more closely related to *D. trypherus*, from which it differs principally in the relative size of the ovary and testes, in the ovary lying very close to the ventral sucker, and in the yolk-glands extending inwards beyond the intestinal limbs in front of the ovary. The follicles of the yolk-glands are about the same size ( $0.055 \times 0.027$  mm.).

This genus appears to be more closely related to *Opisthioglyphe* Looss, than to any others, and its members may be looked upon as Australian representatives of *O. endoloba*, occurring in the intestine of European frogs. It agrees with the genus named in the configuration of the alimentary canal and the excretory vesicle; to a less extent, in the topography of the genital glands and the yolk-glands, in the presence of Laurer's canal, and a receptaculum seminis (?), in the character of the copulatory organs, and in the form and extent of the uterus. *Dolichosaccus* differs from *Opisthioglyphe*, however, in the details of many of these organs, as well as in some

other points. The character of the integument and suckers is very similar in the two genera, but the form of the body is different, being narrower and more pointed in *Dolichosaccus*. The testes are of different shape, and, relative to the ventral sucker, the ovary is placed further back in the Australian genus, while the cirrus-sac is much larger, and Laurer's canal a much more conspicuous structure. The yolk-glands, too, are more extensively developed in the posterior region of the body.

Literature referred to—8, 9, 31, 48, 50, 51, 57, 75, 97.

GENUS BRACHYSACCUS,\* gen. nov.

Subfamily Plagiorchinæ Lühe, (Lepodermatinæ Looss).

(Figures 9, 10, 51-55.)

*Diagnosis*.—Small, *lancet-shaped worms*, with a flattened elliptical cross-section. Integument spiny, the spines disappearing towards the posterior end. Ventral sucker at the beginning of the middle third of the body. Pre-pharynx, pharynx and œsophagus present; intestinal limbs reaching the posterior end of the body. Excretory vesicle Y-shaped, *with the stem running between the testes*. Testes *obliquely one behind the other* in the posterior end of the body. Ovary between the testes and the ventral sucker. Copulatory organs present; genital pore on the ventral surface near the œsophagus, *cirrus-sac wholly in front of the ventral sucker*, and much shorter than in *Dolichosaccus*; *a very long Laurer's canal* and receptaculum present. Yolk-glands lateral, mainly outside the intestinal limbs, *extending from the intestinal fork to near the posterior end* of the body. Coils of the uterus *between the testes and the cirrus-sac*.

In the intestine and rectum of frogs.

Type species, *Brachysaccus anartius*, sp.n.

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\* βραχυσ, short.



## BRACHYSACCUS ANARTIUS,\* sp.n.

(Figures 9, 51-55).

*Diagnosis.*—Small, *lancet-shaped* worms, with a flattened oval cross-section, about 3.3 mm. long, and 1.2 mm. broad. Integument spiny, the spines disappearing towards the posterior end. *Suckers almost equal in size*, but the oral (0.205 mm.) rather smaller than the ventral (0.212 mm. in diameter). Pharynx comparatively large (0.148 mm. in diameter); pre-pharynx well-developed; œsophagus about equal in length to the pharynx; intestinal limbs reaching the *extreme posterior end* of the body. Excretory vesicle Y-shaped, winding between the testes. Genital pore on ventral surface beneath the œsophagus. Testes rounded 0.175 mm. in diameter, lying *one obliquely behind the other* in the middle of the posterior body-third. Ovary oval or rounded, smaller than the testes, 0.142 mm. in diameter, lying to one side of the middle line, about midway between the testes and ventral sucker. Laurer's canal very long. Yolk-glands of numerous small follicles laterally placed, not extending forwards beyond the intestinal fork, nor inwards beyond the intestinal limbs while in front of the testes; but those on the left side *cross over behind the testes, and fill up some of the space* between the testes and the posterior end of the body. Loops of the uterus richly developed, *never reaching behind the testes*, but filling up most of the space *between the testes and the cirrus-sac*. Eggs very numerous, 0.034 × 0.019 mm.

In the intestine and rectum of *Hyla aurea*, and *Limnodynastes peronii*.

Type-specimen in the Australian Museum, No. W.337.

I have obtained over two hundred specimens of this worm from the hosts named. Whilst it is very common in *Hyla aurea*, being found more often than any other trematode in that frog, it is much less common in *L. peronii*, in which I

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\* ἀνάρτιος, uneven, odd, referring to the yolk-glands.

found it only twice. The alimentary canal exhibits the well-developed pre-pharynx characteristic of the group. The spherical pharynx, with its front edge indented, has strong muscular walls, and the œsophagus is equal in length to the pharynx (Fig. 52). The photograph (51) shows the intestinal limbs reaching to the extreme posterior end of the body. The Y-shaped excretory vesicle has a comparatively long stem, which, like that of *Astiotrema* (Looss, 50), winds between the testes, and divides into its two branches just behind the shell-gland (Fig. 54). The cirrus-sac lies wholly in front of the ventral sucker as in *Opisthioglyphe*: the seminal vesicle is constricted in the middle like that of *Dolichosaccus*; the copulatory organs are strongly developed. As regards the location of the gonads, the ovary is generally on the right of the middle line, while the left testis is anterior to the right; but the sexual amphitype is of frequent occurrence, 28 per cent. of the individuals exhibiting this curious variation. Laurer's canal is of extraordinary length. Its course is less tortuous than in *Dolichosaccus*, but it runs right down to near the posterior end, opening on the dorsal surface quite near the blind ends of the intestines (Fig. 53). The extension of the yolk-glands is also peculiar. On the right side, they occupy the space between the lateral body-wall and the intestinal limb, stretching from just behind the intestinal fork back to the level of the posterior testes, and do not anywhere extend inwards beyond the intestinal limb. On the left side, their disposition in front of the testes is exactly the same as that on the right, but they extend further back, and, stretching inwards behind the testes, partly fill up the space lying between those organs and the posterior end of the body. This distribution of the yolk-glands is a very constant character, being exactly similar in every specimen, except that in the sexual amphitype, of course, it is the follicles on the right-hand side that extend beyond the testes.

The coiling of the uterus is very rich and complex, so that, although its calibre is only small, all the body-space between

the cirrus-sac and the testes presents the appearance, in whole mounts, of being stuffed full of eggs, which are themselves comparatively small.

*BRACHYSACCUS SYMMETRUS*,\* sp.n.

(Figure 10).

*Diagnosis*.—Somewhat *lancet-shaped* worms, moderately stout, about 1.47 mm. long, by 0.57 mm. broad. Integument spiny, the spines disappearing towards the posterior end. Oral sucker (0.256 mm.) larger than the ventral (0.215 mm.); *ratio of oral to ventral*, 6:5. Alimentary canal and excretory vesicle as in the genus; but the intestinal limbs do not extend so far back as in *B. anartius*. Testes oval, 0.147 × 0.105 mm., lying very close to one another, and *obliquely one behind the other* in the posterior end of the body. Ovary oval, about same size as the testes (0.147 × 0.101 mm.), long diameter transversely placed, at about the middle of the body-length. Yolk-glands arranged in a *pair of anterior and a pair of posterior masses*. The anterior masses do not extend inwards beyond the intestinal limbs; but the posterior masses stretch under the whole surface, from the level of the testes backwards. Loops of the uterus as in *B. anartius*. Eggs, 0.034 × 0.019 mm.

In the *rectum* of *Hyla cœrulea*.

Type-specimen in the Australian Museum, No. W.338.

This species, I have met with only twice; three individuals were obtained from the rectums of two specimens of *Hyla cœrulea*, one in one rectum and two in the other. Two of the specimens were mounted whole, and the other sectioned. This species differs from *B. anartius* mainly in its much smaller size, and in the relative sizes of the suckers, which are here very distinctive, the oral sucker being smaller than the ventral in *B. anartius*, but larger than it in *B. symmetricus* in having the testes and ovary about the same size, and in the

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\* *συμμετρος*, referring to the yolk-glands.

disposition of the yolk-glands, the respective disposition in each species being very constant and characteristic. In *B. symmetricus*, the two lateral lines of yolk-follicles that occur in *Opisthoglyphe*, *Dolichosaccus*, and *B. anartius* have become broken up into anterior masses and posterior masses. The anterior masses extend from the level of the pharynx backwards to the level of the ventral sucker, and do not extend inwards beyond the intestinal limbs; the posterior masses begin in a lateral position, a little in front of the testes, and gradually spread inwards (Fig. 10), till they spread under the whole posterior surface.

*Brachysaccus* seems to find its nearest relations in *Opisthoglyphe* and *Dolichosaccus*, differing from them mainly in the much more extended disposition of the uterus-loops, and the oblique position of the testes. The great length of Laurer's canal seems to represent the extreme development of elongation, shown in a more moderate degree in *Dolichosaccus*. The character of the integument, the configuration of the alimentary canal and of the excretory vessels, and, with the exception of the uterus, the disposition and character of the genital organs, including the yolk-glands, is in pretty close agreement in all three genera, so that they all evidently belong to the same sub-family.

Literature referred to—8, 9, 31, 48, 50, 51, 57, 75, 97.

#### GENUS PNEUMONŒCES LOOSS.

##### PNEUMONŒCES AUSTRALIS,\* sp.n.

(Figures 11, 56-62).

*Diagnosis*.—Elongated oval worms, narrower in front, 3.5 mm. long by 1.52 mm. broad. Skin smooth, without spines. Oral sucker 0.398 mm., ventral 0.193 mm. in diameter; *ratio of oral to ventral* 2:1. Pharynx equal in size to ventral sucker; œsophagus short; intestinal limbs reaching almost to

\* australis, southern.

posterior end of body. Genital opening on the ventral surface, near the middle line, just behind the oral sucker. Testes very large, *oval, with a few deep grooves*, the posterior testis *longer and narrower than the anterior*; situated in the posterior part of the body, on either side of the middle line, one *a little behind the other*. Cirrus-sac very long and tubular, with a slightly coiled *vesicula seminalis*: a strong cirrus and ejaculatory duct. Ovary *deeply lobed, large and elongated*, laterally placed in front of posterior testis, on a level with the ventral sucker. Receptaculum seminis very large, *on a level with, and ventral to the ovary*. Postero-lateral uterine loops present, *not reaching forward as far as the ovary*. *Yolk-glands in four main groups*, a pair of anterior groups, each of 4 or 5 bunches of 15 follicles, and a pair of posterior groups, each of 5 or 6 bunches of 12-15 follicles. Eggs *very small*,  $0.020 \times 0.014$  mm.

Hosts: *Hyla aurea* and *Limnodynastes peronii*.

Type-specimen in the Australian Museum, No. W.339.

The shape of these worms varies from an elongated oval, narrower in front, to tongue-shaped. Figures 56-57 and 62 are photographs of typical specimens. Leaving out the very small ones, which may be looked upon as more or less immature, the size averages 3.5 mm. long, by 1.52 mm. broad, with a maximum length of 4.9 mm., and a minimum of 2.5 mm. The integument is quite smooth, showing no trace of spines or tubercles. The oral sucker, subterminal in position, is large, while the ventral is small. The relation in size between the suckers is a very constant one, being exactly the same, viz., 2:1, in most of those measured. The average sizes are, oral 0.398 mm., ventral 0.193 mm. The ventral sucker is placed just anterior to the middle of the body-length. The relative size of the pharynx is also very constant: it is globular in form, with a diameter of 0.191 mm., *i.e.*, slightly smaller than the ventral sucker. There is a short but distinct oesophagus, and the wide intestinal limbs run practically to the posterior end of the body.

The main stem of the *excretory system* of vessels extends from the posterior extremity, where the pore opens, forwards to the posterior side of the receptaculum seminis. The terminal part is provided with a well-developed sphincter, and is surrounded by a mass of glandular cells. From the anterior end of the vesicle, two main collecting tubes proceed forwards as far as the intestinal fork. These tubes are of somewhat varying calibre, but, on the whole, are nearly as wide as the vesicle itself. In the first part of their course, they remain closely applied to the sides of the receptaculum seminis, but at its anterior border, they pass outwards till they reach the outer aspect of the intestinal limbs, along which they run to their anterior end. All along their course, they give off smaller vessels (Figs. 60, 61, 62), the branches of which end in flame-cells. The *testes* are large, and are situated in the posterior half of the body, rather nearer the ventral sucker than the posterior end. They lie, one on either side of the middle line, one slightly or sometimes more markedly more anterior than the other (Figs. 11, 57). The form is oval or a little more elongated, with the outline, in most cases, but not invariably, marked by a few deep indentations. The posterior testis is invariably longer and narrower than the other: the average measurements are, anterior  $0.529 \times 0.379$  mm., posterior  $0.668 \times 0.346$  mm. The vasa deferentia come off from their anterior ends, and join to form the vesicula seminalis, a little in front of the ventral sucker. The cirrus-sac is tubular and very long, in the extended animal being practically straight, while in preserved specimens it shows one or two gentle curves. The vesicula seminalis within this, is slightly coiled at each end (Figs. 59, 60). The prostate is only poorly developed, but the cirrus is well developed, with a strong ejaculatory duct (Fig. 58). The male duct opens into a distinct genital chamber, and the genital opening is situated on the ventral surface, about the middle line, just beneath the pharynx (Fig. 58).

The *ovary* is elongate, convex on its outer surface, concave on the inner, and divided up into five or six deeply marked

lobes. It is situated close under the dorsal surface (Fig. 59), on a level with the ventral sucker and to one side of it, normally on the right side, but in ten per cent. of the specimens, it lies on the left side. In any case, it lies on the same side as the posterior testis. In the amphitype, not only are the ovary and posterior testis placed on the left side, but the first postero-lateral uterine loop runs on the right side instead of the left. The oviduct comes off from the inner surface of the ovary, and runs towards the middle line, being very soon joined by a canal coming from the enormous receptaculum seminis (Fig. 61). Immediately after this, it is surrounded by the cells of the shell-gland, a comparatively large mass of cells lying to one side of the ovary: and in this part of its course, it also receives the main duct of the yolk-glands. The receptaculum seminis, of relatively enormous size, up to 0.68 mm. in diameter, is pretty circular in ventral view, and lies on the same level as the ovary and ventral to it, with the ventral sucker generally about its middle. There is no Laurer's canal. In the next part of its course, the uterus is generally filled with spermatozoa, and this portion may be looked upon as the receptaculum seminis uterinum. Then, as the uterus, it runs backwards to the extreme posterior end of the body as a median descending loop, which is sometimes pretty straight, but, in most older specimens, exhibits several spiral coils, some of which, in front of the testes, reach the lateral margins of the body (Fig. 57). On reaching the posterior end, the characteristic postero-lateral loops run along the margins of the body, first to the left, then to the right. These postero-lateral loops do not reach as far forwards as the ovary, and, in old specimens, tend to be coiled (Fig. 11). At the end of the right, descending, postero-lateral loop, the uterus turns forwards again in the middle line, forming the median ascending loop, which, as far as the ventral sucker, closely resembles the form of the descending median loop. In front of the ventral sucker, in a number of closely placed transverse coils, which generally extend to the outer borders of the

intestinal limbs, it proceeds forwards to the genital chamber. Both the descending and ascending median loops pass between the ovary and the anterior testes, and between the two testes, and the uterus lies, for the most part, near the ventral surface of the body.

The *yolk-glands* consist of a large number of oval follicles, about  $0.085 \times 0.05$  mm. in size, placed close to the dorsal surface (Figs. 59, 60). Their arrangement into groups is characteristic. A pair of anterior patches extend forwards as far as the fork of the intestine, and from the lateral margin inwards nearly to the middle line (Fig. 56), and backwards as far as the ventral sucker. Each patch is made up of four or five groups of 14 or 15 follicles each. The common duct of each group joins the main anterior lateral duct on each side. Each pair of posterior patches consists each of six groups of follicles, each group containing 12-15 follicles; they are more laterally confined than the anterior patches, and only pass inwards beyond the inner border of the intestinal limbs at their extreme posterior end, and that generally only on one side (Fig. 56). These posterior patches extend from behind the ovary, almost to the ends of the intestinal limbs (Fig. 56). The common ducts of each group join a posterior lateral duct on each side. The anterior and posterior lateral ducts meet about the middle of the body, to form right and left transverse ducts which unite, near the middle line, to form the final part of the duct that opens into the ootype.

The *eggs are extraordinarily numerous*, very uniform in size, and very small,  $0.02 \times 0.014$  mm.

This species of worm occurs in the lungs of *Hyla aurea* and *Limnodynastes peronii*, and these are the only frogs in which I have found trematodes in the lungs. These lung-flukes do not appear to be so common in Australian frogs as they are in European (Hollack, 33), and American frogs (Stafford, 88). nor, when they do occur, are they present in such large numbers. I have never found more than 10 in one frog, and, as a rule, only one, two or three are found. Only two of my



specimens have been found in *Limnodynastes*, and I think there is no doubt that they are identical with those from *Hyla*, of which I have 59 specimens, ten of which have been sectioned. This fluke furnishes specially beautiful whole-mounts, stained either with safranin or hæmatoxylin, and decolourised till the parenchyma, from which these stains wash out more easily than from the other tissues, has only a faint tinge of colour.

On comparison with the other members of this genus, *Pneumonæces australis* shows close similarities with *P. capyristes* Klein, and *P. variegatus* R., being more nearly related in its structure to the former. Amongst those characters in which specific differences occur in this genus, it agrees with both species mentioned in the smooth integument, in the antero-posterior extension of the yolk-glands, in the position of the gonads, and, on the whole, in the arrangement of the uterine loops. It further agrees with *P. variegatus* in its lobed ovary and elongated tubular cirrus-sac (Looss, 48), differing from it, however, in the form of the testes, and in the arrangement of the follicles of the yolk-glands, in the relative size of the suckers, and in the much smaller size of the eggs, as well as in several minor points. It further agrees with *P. capyristes* Klein, in the form of the testes, especially in the larger size of the posterior (Klein, 38); the arrangement of the uterine loops, and the position of the ovary and related parts correspond more closely to Klein's species than to Rudolphi's; our Australian species differs from *P. capyristes*, however, in the shape of the ovary and cirrus-sac, in the arrangement of the follicles of the yolk-glands, and in the size of the eggs.

Literature referred to—14, 38, 48, 50, 51, 55, 79, 81, 88.

Genus GORGODERA LOOSS.

GORGODERA AUSTRALIENSIS, sp.n.

(Figures 12, 63-68.)

*Diagnosis*.—Elongated, oval worms with the usual narrower, very extensible portion anterior to the ventral sucker,

and a thicker, wider and less mobile division behind it. Size moderate, average length 5.7 mm., by 1.76 mm. broad. Integument smooth, without spines or tubercles. Oral sucker 0.38 mm. in diameter, ventral 0.589 mm.; ratio of oral to ventral, 2:3. Genital opening just behind the intestinal fork. Testes irregular in shape and lobed, in two antero-posterior rows, five on one side and four on the other. Vesicula seminalis large; no cirrus-sac. Ovary lobed, large (0.375 mm. long), always in line with the five testes. Laurer's canal present, but no receptaculum seminis; a very large receptaculum seminis uterinum. Uterus of small calibre, but very long, with very numerous coils that extend out to the lateral edges of the body. Yolk-glands a pair of compact bodies of from three to six close-lying lobes. Size of eggs 0.033 mm.  $\times$  0.019 mm.

Hosts, *Hyla aurea* and *Limnodynastes peronii*, in bladder.

Type-specimen in the Australian Museum, No. W.340.

Elongated worms with the narrower, very mobile and extensible anterior portion in front of the ventral sucker, and a less mobile, wider and thicker posterior part behind it, as in *Gorgoderia* generally.

The average length is 5.7 mm., with a maximum of 9.95 mm., and a minimum of 3.96 mm., average breadth 1.76 mm. Integument smooth, without spines or tubercles. The oral sucker is subterminal and rounded, with an average diameter of 0.38 mm. The ventral is a large, globular sucker, deeply implanted in the body (Fig. 65), with an average diameter of 0.589 mm. Ratio of oral to ventral sucker, 2:3. The sucker does not, as a rule, extend laterally beyond the intestinal limbs; the opening of the oral sucker is on the ventral surface; the excretory pore is terminal and posterior, and the genital opening in the middle line, halfway between the ventral and the oral suckers, just behind the fork of the intestine. As in *G. cygnoides*, there is a small group of glands (the "head glands") on each side just behind the oral sucker.

In the alimentary canal, there is no muscular pharynx; the œsophagus is moderately long (Fig. 63), with walls of fairly

even thickness; the intestinal limbs joining the base of the œsophagus, reach right up to the posterior end of the body. As a rule, they curve round the ventral sucker, passing quite lateral to it; but sometimes one of the limbs passes dorsal rather than lateral to it.

The *nervous system* and *excretory system* closely resemble what is described by Looss for *D. cygnoides* in "Dist. unserer Fische u. Frösche"; but I have not attempted to count the number of flame-cells. The excretory vesicle runs as a single tube in the middle line, near the dorsal surface, and between the lines of testes, as far forward as the level of the middle of the ovary, where it divides into two branches that diverge, and extend anteriorly up to the oral sucker. The *flame-cells* have a form very similar to that described and figured by Looss (48).

*Genital Organs.*—The testes, more or less rounded bodies, with deep grooves or indentations on the surface (Fig. 64), are nine in number, five on one side and four on the other, joined together in two series, in the characteristic way, by a tube passing from one to the next in front. The vasa deferentia unite near the anterior edge of the ventral sucker, to form a very short common tube, 0.048 mm. long, before opening into the base of the vesicula seminalis; in *G. cygnoides* they unite much further back (just in front of the yolk-glands), and have a considerable course as a single tube before entering the vesicula seminalis (Looss 48). The vesicula seminalis (Fig. 65) is a shortly coiled tube, very wide posteriorly, when filled with sperms, suddenly narrowing to the ejaculatory duct, which runs straight to the genital atrium, a narrow space opening on the ventral surface (Fig. 65). There is no cirrus-sac, but the vesicula seminalis is surrounded by a layer of slightly modified parenchyma-cells; the prostate is represented by a very few cells lying near the junction of the ejaculatory duct and the vesicula seminalis. The ovary lies in front of the line of five testes, generally on the left side; but, as in the other species of this genus, the sexual amph-

type is very common, for in 38 cases out of 86 worms examined for this character, the five testes and the ovary lie on the right side instead of the left. The ovary is marked off into several lobes (generally four), by comparatively shallow grooves. The oviduct, after a short course to the side, turns and runs towards the middle line, at the same time widening out somewhat to form the fertilisation-space; reduced again to a smaller calibre, for a short distance, it soon widens again to form the ootype, at the commencement of which the short Laurer's canal is given off, which, after a very short, direct course, opens on the dorsal surface (Fig. 66). As is usual in *Gorgodera*, there is no receptaculum seminis. The yolk-reservoir, given off from the short, transverse, vitelline duct, opens into the distal end of the ootype. The *shell-gland* takes the form of a rounded mass of cells lying on one side of the ootype, in the space between the anterior ends of the yolk-glands. The receptaculum seminis uterinum, the next division of the female duct, is a more than ordinarily large section, and occupies a number of coils that lie partly in the middle of the body, and partly laterally, outside the intestine on one side (Fig. 68). The coils of the uterus are narrow, but very numerous, running down the middle near the ventral surface, and ascending again nearer the dorsal surface, both ascending and descending limbs sending out, laterally, loops between the testes, each loop extending out to the edge of the body, and forming a coil of several turns in the space between the testes and the lateral edge of the body (Fig. 67). The ascending coil having reached the region in front of the ovary and behind the vesicula seminalis, makes several transverse turns almost right across the body (Fig. 64), then runs forward behind and partly to the side of the ventral sucker, the final part of its course lying parallel to the ejaculatory duct. The yolk-glands take the usual form of a pair of compact glands lying in front of the ovary, joined by a wide transverse duct, each gland being composed of 3-6 well-marked lobes.

*Eggs*.—The oldest eggs in the uterus, situated in the coils just behind the ventral sucker, and in the loop passing forwards to the genital opening, with perfectly smooth and un-wrinkled surface and full contents, show no sign of shrinkage, and measure  $0.033 \times 0.0214$  mm.

*Gorgoderia australiensis* is found in the bladder of *Hyla aurea* and of *Limnodynastes peronii*. It is rather commoner in the Hyla, 75 of my mounted specimens coming from that frog, as against 23 from *Limnodynastes*. Sometimes only two or three worms will be found in the same bladder, but more generally one finds ten or a dozen.

The Australian species differs from the European *G. cygnoides* in its smaller size, and in the relatively smaller size of the ventral sucker, in the shape of the ovary and its position in regard to the testes, and in the length of the united portion of the vas deferens, as well as in the size of the eggs. It further differs from the American species, *G. amplicava*, in size (the largest *G. amplicava* being smaller than the average Australian form), and in the proportional size of the suckers. The ratios of oral to ventral sucker in *G. australiensis*, *G. cygnoides*, and *G. amplicava* are respectively, 2:3, 2:4, 2:5 (Stafford, 89), or 2:6 (Bensley, 3).

Ssinitzin's paper on the trematodes of frogs near Warsaw (84), in which he makes four species of European trematodes belonging to the genus *Gorgoderia*, I have unfortunately not been able to see.

Literature—3, 8, 42, 48, 50, 52, 59, 67, 68, 71, 72, 84, 89.

### Genus M E S O C Œ L I U M Odhnr.

Subfamily **Brachycoeliinæ** Looss.

(Figures 13, 14, 15, 69-76.)

*Diagnosis*.—Small, somewhat oval flukes, *more or less spiny*, oral sucker the larger, with pharynx, moderately long œsophagus, and short *intestinal limbs reaching to, or a little beyond, the middle of the body*. Excretory vesicle tubular and long,

at the anterior end marked off into two very shallow pockets. Testes entire, *symmetrically placed on either side of the middle line*, near and mostly behind the ventral sucker; ovary entire, *in a variable position behind the testes*; vesicula seminalis, cirrus-sac, prostate, and penis moderately or well-developed; genital opening in the middle line, between the suckers; receptaculum seminis and Laurer's canal present. Yolk-glands a single group of rounded follicles on either side, in the anterior part of the body, *not passing inwards beyond the intestinal limbs*. Coils of the uterus in transverse loops, filling up most of the body-space behind the gonads, only in the terminal part passing in front of these.

In the intestine of frogs (Anura).

MESOCOELIUM MESEMBRINUM, \* sp.n.

(Figures 13, 69-76.)

*Diagnosis*.—Moderately small worms, length 2.3 mm. Oral sucker the larger, ratio of its diameter to that of the ventral, 3:2. Body closely covered with small, backwardly directed spines, *gradually becoming fewer and smaller up to the posterior end*. Pharynx well developed; *intestinal limbs reaching the middle of the body*. Excretory vesicle tubular, reaching up to the shell-gland, inconspicuously divided in its anterior part. Testes symmetrically placed near the ventral sucker; *ovary behind the right testis*; copulatory organs moderately well developed. Genital opening in the middle line, halfway between pharynx and ventral sucker. Receptaculum seminis and Laurer's canal present. Uterus filling up the posterior part of the body. Yolk-glands laterally placed in the anterior part of the body, *not passing inwards beyond the intestinal limbs, nor backwards beyond the ovary*. Eggs thick-shelled, light brown at first, becoming dark brown, 0.040 mm. long, and 0.025 mm. broad.

Host, *Hyla carulea*, in the duodenum.

\* μεσικοελίου, southern; referring to its geographical distribution.

Type-specimen in the Australian Museum, No. W.341.

In the duodenum of our green tree-frog, *Hyla caerulea*, I found specimens of this fluke three times, getting four specimens from one frog in the winter, 85 and 7 from two others in the summer, making a total of 96 flukes, 25 of which were cut into sections, and the remainder mounted as whole-mounts. Those found in the first case, were somewhat larger than the others, their length varying from 2.44 to 3.7 mm., their breadth 0.89-1.6 mm., average length 3.14 mm., and breadth 1.19 mm., while the average measurements of the others are 2.035 mm. long, by 0.644 mm. broad. The suckers, in the larger specimens, are naturally rather larger than in the smaller ones, the oral sucker being 0.2934 mm. in diameter, the ventral sucker 0.1956 mm., the ratio of oral to ventral being 1.5:1. About the same ratio between the suckers holds for the smaller specimens, and, in the rest of their organisation, they are practically identical with the larger. The shape of the living worms was fusiform, both ends being about equally pointed; but the anterior end is much more mobile, and when the worm is killed, generally contracts more, so that, in preserved specimens, the anterior end is more bluntly rounded off than the posterior. When the body-length is marked out into fourths, the ventral sucker lies at the beginning of the second quarter.

The body is covered by small, backwardly directed spines, densely arranged on the anterior part of the body, and gradually becoming fewer towards the posterior end. The spines are narrow and pointed, wider and thicker at the base, regularly arranged in transverse rows, 0.0115 mm. long, 0.0023 mm. broad, 0.0092 mm. apart at the level of the gonads. They are present both on the dorsal and ventral surfaces, right up to the posterior end, differing in this respect from *Distomum sociale* Lühe, where, on the dorsal surface, they cease about the middle of the body (Lühe 60). The opening of the oral sucker in the living worm is terminal or slightly subterminal: the excretory pore is at the extreme posterior end; the genital

aperture is in the middle line on the ventral surface, midway between the oral and ventral suckers.

*Alimentary Canal.*—The pharynx, lying at the base of the oral sucker is rounded and thick-walled, 0.11 mm. in diameter. It is followed by a moderately long, thin-walled œsophagus, which, at its posterior end, becomes pretty wide. The intestinal limbs are short, reaching to the middle of the body, some distance beyond the ovary.

*Nervous System.*—There is a pair of nerve-ganglia, one on either side of the posterior end of the oral sucker, joined by a thick, transverse commissure over the dorsal aspect of the pharynx, with fine nerves given off anteriorly and posteriorly.

*Excretory System.*—The excretory vesicle is tubular, somewhat dilated near its posterior end (Fig. 70), reaching in front to just behind the shell-gland and receptaculum seminis, where it ends in two shallow pockets (Fig. 71). It gives off, in its course, a few fine tubes; and two, main, fine tubes enter the anterior end, one to each pocket. The vesicle is lined throughout by glandular cells, which project into its lumen.

*Reproductive System.*—The testes vary in shape, from round to oval, measuring about 0.195 mm. in length, or rather less in diameter, when round in shape. The ovary, which is rounded, is a little smaller than the testes (0.163 mm. in diameter), but in a few cases, where the structure of the testes does not appear quite normal, the testes are smaller than the ovary. The testes vary a little in position; they are more or less symmetrically placed, dorsal and lateral, or somewhat posterior to the ventral sucker (Fig. 69). The ovary is placed behind the right testis, differing in this from *D. sociale* Lühe, where it lies behind the left testis. In two cases, however, there is a "situs inversus" or sexual amphitype (4, 5, 32, 36, 39, 55), and the ovary lies behind the left testis instead of the right.

The vasa deferentia are short (Fig. 74), entering the base of the vesicula seminalis together; the latter is a coiled tube,



generally of two turns, lying in the base of the cirrus-sac. The ejaculatory duct (Fig. 73) opens out of the anterior end of the vesicula seminalis, and traverses the penis, which is fairly well developed. The cirrus-sac is well developed, with muscular walls; the prostate is represented by glandular cells lying in the parenchyma, within the cirrus-sac.

The *oviduct*, passing off from the inner side of the ovary, runs towards the left side of the body, and has connected with it, in this part of its course, the receptaculum seminis, Laurer's canal, the yolk-duct, and the shell-gland, in the order named, beginning at the ovary. The receptaculum seminis is rather small, in all the specimens sectioned containing sperms; Laurer's canal is short, running almost as a straight tube to the dorsal surface, where it opens near the middle line. The shell-gland, or gland of Mehlis, is a compact mass of large glandular cells surrounding the oviduct, near the place of opening of the yolk-duct, just beyond the exit of Laurer's canal.

The *uterus* passes down to the extreme posterior end of the body, in a number of coils passing transversely across the body, and lying near the dorsal surface, ascending in the same manner by a number of transverse loops lying near the ventral surface to the left side of the ventral sucker, whence it runs forwards to the genital opening, in a course parallel to the cirrus-sac. The terminal part, the metraterm or vagina, is provided with well-marked, muscular walls (Figs. 72, 74). The loops of the uterus fill up all the body-space behind the genital glands, not occupied by the intestine and excretory vesicle. The follicles of the yolk-glands (Fig. 72) are moderately large ( $0.084 \times 0.035$  mm.), oval or pear-shaped, and occur in a triangular patch, on each side, in the anterior part of the body. There are about 35 follicles in each group. They do not extend inwards beyond the intestinal limbs: anteriorly they extend as far forwards as the posterior border of the oral sucker, whilst posteriorly they do not reach beyond the ovary, generally not so far as its anterior border. In the sections, the

yolk-follicles may be seen to be loaded with droplets of the material that goes to form the shell (Goldschmidt 26).

The thick-shelled eggs (0.0022 mm. thick) are oval in shape, 0.04 mm. long, by 0.025 mm. broad, are very numerous, and, at first, light brown in colour, gradually becoming very much darker.

This worm is evidently closely related to *Distomum sociale* Lühe, having the same generic characters, and differing from it mainly in the degree of spininess of the body, in the relative size of the suckers, in the rounded instead of triangular shape of the gonads, in the right instead of left position of the ovary, and in the less elongated extension of the yolk-glands. I shall have more to say about its relationships, a little later.

MESOCÆLIUM MEGALON,\* sp.n.

(Figure 15.)

*Diagnosis.*—Habit slender and delicate, elongated oval, broader behind, 1.8 mm. long, and 0.358 mm. broad at the widest part; *integument smooth, without spines*; oral sucker 0.1935 mm. in diameter, ventral 0.0645; *ratio of oral to ventral, 3:1*. Oesophagus moderately long, intestinal limbs short, hardly reaching beyond the ovary. Testes oval, (0.181 × 0.129 mm.), placed dorsally and laterally in regard to the oral sucker, and extending backwards behind it; ovary spherical, 0.112 mm. in diameter, *not quite lateral but latero-median, more on the left side*. Yolk-glands with rounded to oval follicles, from 0.017 to 0.034 mm. in diameter, comparatively numerous, about 70 on each side; the two lateral groups extending near the dorsal surface across the body to the middle line, but *posteriorly not extending beyond the ovary*.

Eggs comparatively large and thick-shelled, average length 0.047 mm., varying from 0.043-0.052 mm., by 0.029 mm. broad.

\* μέγας, large; ὄον, egg.

Host, *Hyla ewingii*, in the duodenum.

Type-specimen in the Australian Museum, No. W.343.

Thirty-two specimens of *Hyla ewingii*, a frog fairly common in the neighbourhood of Sydney, yielded a single specimen of this fluke, which occurred in the duodenum. It is a thin, delicate worm, with the posterior end distended with eggs; and, in the process of mounting, it became broken in two, just where the coils of the uterus, crowded with eggs, begin to fill up the body. At first, I intended not to describe it till more material became available, but the interesting variation it shows in the position of the ovary, combined with the other well-marked specific characters, decided me to publish a description of it with the others now, in order to make the group more complete.

#### MESOCÆLIUM OLIGOON, sp.n.

(Figure 14.)

*Diagnosis*.—Small, delicate worms, oval, 1.53 mm. long, by 0.59 mm. broad; body covered with *very small spines, comparatively few in number*; oral sucker subterminal, 0.215 mm. in diameter, ventral sucker at the junction of the anterior and middle body-thirds, small, 0.129 mm. in diameter; *ratio of oral to ventral sucker, 5:3*; pharynx globular (0.065 mm.); intestinal limbs short, of unequal length. Testes polyhedral, large, 0.165 × 0.099 mm., about one-ninth of the body-length; ovary oval, with smooth contour, large (0.133 × 0.09 mm.), *situated behind the right testis*. Yolk-follicles rounded to oval, 0.028-0.052 mm. long, comparatively numerous, about 70 on each side, closely arranged in a somewhat triangular group on each side of the œsophagus, *extending backwards laterally to beyond the intestinal limbs*. Eggs few in number, comparatively large, 0.039-0.052 mm. long, by 0.028 mm. broad, average length

\* ὀλίγος, few; ᾠον, egg.

0.044 mm., thick-shelled, with, at the narrower end, a much thickened patch of shell, that is often developed into a blunt process.

Host, *Hyla citropus*, in the duodenum.

Type-specimen in the Australian Museum, No. W.342.

In November last, I collected at the Lodden River, near Bulli, N.S.W., two specimens of *Hyla citropus*, a rather rare frog, and from the intestine of one of these, I obtained two small flukes, described above. One of the two was smaller than the other, and hardly sexually mature.

### Genus MESOCOELIUM Odhn.

#### Table for determination of the Species.

- Integument densely spiny; ratio of oral to ventral sucker not greater than 3 : 2.
- Testes triangular; ovary triangular, behind left testis; yolk-glands extending backwards to behind the ovary; eggs  $0.040 \times 0.025$  mm. .... *M. sociale*.
- Testes oval or rounded; ovary oval or rounded, behind the right testis; yolk-glands not extending backwards beyond the ovary; eggs  $0.04 \times 0.025$  mm. .... *M. mesembrinum*.
- Integument smooth or very little spiny; ratio of oral to ventral sucker greater than 3 : 2.
- Testes oval; ovary spherical, latero-median; yolk-glands not extending backwards behind ovary; eggs  $0.047 \times 0.029$  mm. .... *M. megaloon*.
- Testes polyhedral; ovary oval, behind right testis; yolk-glands extending backwards behind ovary; eggs  $0.044 \times 0.028$  mm. .... *M. oligoon*.

Amongst known distomes, it is to *Brachycœlium crassicolle* R., that the four species of this genus appear to be most closely related, and I think they may be looked upon as the Asiatic and Australian representatives of that worm. I place the genus in the subfamily Brachycœliinæ, in which Looss (50) places *Phaneropsolus*, *Lecithodendrium*, *Pycnoporos*, and *Brachycœlium*. Pratt, in his Synopsis of American Invertebrates (75) also places the same genera in this subfamily. In the characters of the integument, the position of the geni-

tal pore, the disposition of the genital glands, the character of the cirrus-sac and related parts,\* the presence of a receptaculum seminis and Laurer's canal, and the disposition of the yolk-glands and the uterine loops, it agrees with the subfamily as defined by Looss(50, p.607), the only important differences being in the relatively considerably longer, though still short, intestinal limbs in *Mesocœlium*, and its tubular excretory vesicle, the Brachycœliinæ being stated to have a V-shaped excretory vesicle. But the excretory vesicle of *M. mesembrinum*, in which species I have studied it most closely, is exactly of the type of vesicle described by Looss (55, p.815) for *Brachycœlium crassicolle*, with its incipient bifurcation at its anterior end (Fig.71).

In other respects besides this excretory vesicle, *e.g.*, in the characters of the cirrus-sac and related parts, in the character of the skin and suckers, position of the genital pore, disposition of the genital organs, etc., *Mesocœlium* appears to be more closely related to *Brachycœlium crassicolle* than to the other members of the suborder. I have been in doubt whether, on account of the character of its excretory vesicle (the relative length of the intestine is not of much importance in discussing differences of subfamily rank), *Mesocœlium* can properly be included with the Brachycœliinæ as defined by Looss, but it must certainly be placed very close to *Brachycœlium crassicolle*, and go into the same subfamily with

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\* Lühe, in his account of *Distomum sociale* (60, pp.171-173), does not mention the cirrus-sac and related parts, though he says in a footnote: "Dass *Lecithodendrium crassicolle* einen typischen, muskulösen Cirrusbeutel besitzt, ähnlich demjenigen der Dicrocoelien, habe ich kürzlich auch noch an lebend untersuchten Exemplaren sicherstellen können." But in all its structures described, *D. sociale* is so closely related to the three species described as new in this paper, that I am taking it for granted that the structure of those parts also closely corresponds to that of its Australian representatives.

it. While there is by no means unanimity\*(57, 58, 60, 98) in placing *crassicolle* in the genus *Brachycaelium* as its generic type, though I do not know the worm from my own personal observation, I feel compelled to follow Stiles and Hassall (95), Looss (55), Stafford (91), and others, in regarding it as the type of *Brachycaelium*.

Lühe, in discussing the relationships of his *Distomum sociale*, says (Zwei neue Distomen aus indischen Anuren, p.173): "Es kann keinem Zweifel unterliegen, dass die vorstehend beschriebene Art mit *Distomum mutabile* und mit den Dicrocælien nahe verwandt ist. . . . . In der That zeigt *Distomum sociale* ausser zu *Distomum mutabile* und den Dicrocælien auch zu den Lecithodendrien, speziell, zu *Lecithodendrium crassicolle*, ziemlich nahe verwandtschaftliche Beziehungen. Die Unterschiede des *Distomum sociale* aus dem Dünndarme der indischen Kröte gegenüber dem im Dünndarme einheimischer Amphibien schmarotzenden *Lecithodendrium crassicolle* bestehen im wesentlichen in der erheblich grösseren Länge der Darmschenkel, der Lage des Keimstockes hinter den Hoden und dem Umstande, dass wie die Darmschenkel, so auch die Dotterstöcke weiter nach hinten reichen."

The *Dicrocæliinæ* (Looss, 50) are a subfamily certainly not very distantly related to the *Brachycaeliinæ*, the main points of difference being in the excretory vesicle, tubular in the former, V-shaped in the latter: in the length of the intestinal limbs, long in the former, very short in the latter: in the invariable presence of copulatory organs in the former, while they are sometimes absent in the latter: in the position of the gonads, and the position and extension of the yolk-gland; and in the larger size of the eggs in the former. Of all the *Brachycaeliinæ*, the various species of *Mesocælium* appear to approach more closely to the *Dicrocæliinæ* than any others of their subfamily, with their practically tubular ex-

\* I have noticed later that Lühe in his "Handbook of Practical Parasitology(1910)," written in collaboration with Braun, accepts *Brachycaelium crassicolle* (p.130, English edition), where a clear figure of the worm is given.

cretory vesicle, moderately developed copulatory organs, and in the larger size (in two species at least) of their eggs, while their intestinal limbs have a length midway between that of a typical brachycœline and a typical dicrocœline. *Mesocœlium*, then, may be looked upon as a genus of the subfamily Brachycœliinæ, which brings that subfamily into closer relationship with the Dicrocœliinæ than was formerly estimated.

Literature referred to.—4, 6, 10, 14, 17, 18, 40, 48, 50, 55, 57, 58, 60, 62, 75, 79, 85, 90, 91, 95, 97, 98.

After the above was written, I had an opportunity, through the kindness of Mr. J. J. Fletcher, the Secretary of the Society, of perusing Dr. Odhner's "Nordostafrikanische Trematoden," published in the Results of the Swedish Zoological Expedition to Egypt and the White Nile. I then found that Dr. Odhner had proposed the generic name *Mesocœlium* for Lühe's *Distomum sociale*; in my paper, I had intended to propose a name referring to the variable position of the ovary for this genus, but as Dr. Odhner's name has priority, I have been able to make use of it. While, from an anatomical point of view, the name *Mesocœlium* is a very suitable one, it seems a pity that a word should be chosen which differs only in its termination from *Mesocœla*, established by Barrin, in 1885, for a genus of animals [Zool. Anz. viii., p. 258].

I cannot agree with Odhner, however, in placing this genus in the subfamily Dicrocœliinæ. For the purpose of comparison, I quote, in parallel columns, on the left and right respectively, Odhner's diagnosis of the subfamilies Dicrocœliinæ and Brachycœliinæ, given on pages 88 and 89 of his work (107); the words in square brackets are remarks of my own. In the middle column, I give the characters of the same parts in *Mesocœlium*. A comparison, too, of my own and Lühe's figures of various species of *Mesocœlium* with Odhner's figure of *Brachycœlium crassicolle* (107, p.91), will show the striking resemblance they all bear to one another.

*Dicrocoelinae* Les.

Spiniess of integument generally wanting. [Only spiny in *Mesocotilium* and *Hoploderma*.]

Ventral sucker mostly the larger. [Not so in *Mesocotilium* and *Hoploderma*.]

Limbs of intestine at least  $\frac{2}{3}$  length of the body.

Testes behind or next to the ventral sucker, symmetrical or oblique or one behind the other.

Genital pore under the fork of the intestine.

Ovary behind the testes.

Yolk-glands extending back behind the testes, and not reaching beyond the ventral sucker in front.

In the liver and gall-bladder of mammals, birds and reptiles [only *Mesocotilium* in Amphibia].

*Mesocotilium* Odhn.

Integument spiny.

Oral sucker the larger.

Limbs of intestine about  $\frac{1}{2}$  the length of the body.

Testes behind or near the ventral sucker, more or less symmetrically placed.

Genital pore in front of the intestinal fork.

Ovary behind the testes, very variable in position.

Yolk-glands chiefly in front of the ventral sucker, meeting in the middle line near the oesophagus.

In the intestine of Amphibia.

*Brachycoelinae* Odhn., nec Lss.

Integument spiny.

Oral sucker the larger.

Limbs of intestine very short, scarcely reaching beyond the ventral sucker.

Testes behind the ventral sucker, more or less symmetrically placed.

Genital pore just in front of the ventral sucker.

Ovary in front of the testes.

Yolk-glands extending up to the pharynx in front, and not beyond the testes behind, and extending towards the middle line near the oesophagus (107, p. 92).

In the intestine of Amphibia.



The above columns show that *Mesocelium* agrees with the *Dicrocœliinæ* in the position of the ovary behind the testes. But in these worms, as pointed out above, the position of the ovary is very variable, and cannot be looked upon as of dominant importance in referring the genus to its subfamily. The position of the genital pore in the median plane is different, but not strikingly different, in the two subfamilies in question as well as in *Mesocelium*, while the length of the intestinal limbs in *Mesocelium*, is intermediate between that in the *Dicrocœliinæ* and the *Brachycœliinæ*. In the character of the integument, in the relative size of the suckers, in the character and disposition of the testes, and in the disposition of the yolk-glands, *Mesocelium* agrees with the *Brachycœliinæ* rather than with the *Dicrocœliinæ*. And, lastly, let us look at the hosts of these worms. *Mesocelium*, occurring in the intestine of Amphibia, is likely, when the above characters are taken into consideration, to be more closely related to the *Brachycœliinæ*, occurring in the intestine of Amphibia, than to the *Dicrocœliinæ*, occurring, as they do, for the most part, in the liver and gall-bladder of mammals and birds.

In regard to the position of *Hoploderma* Cohn, I am inclined to think, from the evidence available, that it should be placed along with *Brachycelium* and *Mesocelium* in the *Brachycœliinæ*. But one should not lay too much stress on such unsatisfactory material as that furnished by worms taken from old spirit-specimens.

Genus PLEUROGENES Looss, 1896 b.

PLEUROGENES FREYCINETI, sp.n.

(Figures 16, 77-80.)

*Diagnosis*.—Body oval, narrower end anterior, 1.45 mm. long, 0.89 mm. broad; oral sucker subterminal, 0.202 mm. in diameter, *ventral sucker in middle of body*, 0.176 mm. in diameter; *ratio of former to latter*, 7 : 6. Body covered by flat spines or scales with their points bluntly rounded, *number and size hardly less at*

*posterior end of body.* Pharynx well-developed, spherical, 0.064 mm. in diameter; œsophagus moderately short; intestinal limbs short, ending alongside the testes, some distance in front of the ventral sucker. Excretory vesicle large, V-shaped, its limbs ending anteriorly just behind the testes; excretory pore terminal. Testes large, laterally placed, *just anterior to ventral sucker.* Cirrus-sac very large, in front of the testes. Genital pore alongside the oral sucker. Ovary near and partly in front of the right testes; *vitelline glands few and rather large,* and in front of the intestinal limbs; loops of the uterus without a second loop crossing the middle line. Eggs small, yellow and brown, rather elongated, flattened, elliptical, very numerous, 0.0236 mm. long by 0.0117 broad.

Host, *Hyla freycineti*, in the duodenum.

Type-specimen in the Australian Museum, No.W.344.

This species was found in the duodenum of *Hyla freycincti*, a not very common frog found in the Sydney district. I met with it only once in 35 frogs examined, and obtained seven specimens. Of these, four were prepared as whole-mounts, and three cut into sections, two having been fixed in Flemming, and five in sublimate acetic. They were all very nearly of the same size, and the size of the mounted specimens is about the same as that of the living, in a state of rest. All the measurements given represent the average measurements of the four mounted specimens, and the parts concerned are so nearly alike in size, that the figures very nearly fit any one of the specimens. The specimens mounted whole were very slightly flattened by the vaseline-method mentioned above, and even this slight pressure caused the penis to protrude a little in every case (Fig.16).

The *body*, which is comparatively thick, presents an oval outline, narrower anteriorly, wider and more bluntly rounded off behind. The oral sucker is larger than the ventral, the ratio being 7:6; and while the former is subterminal, with its opening directed ventrally, the latter is situated in the middle of the body. The body is covered by rows of close-set,

flat spines or scales, which have their free ends bluntly rounded and directed posteriorly (Fig.80). Each scale is 0.01 mm. broad at its base, and 0.01 mm. long, and the rows are 0.01 mm. apart all over the body. At the posterior end, the scales are a little shorter, and not quite so close-set.

The *pharynx* is globular, 0.065 mm. in diameter, with thick muscular wall; the œsophagus is narrow, but fairly thick-walled, and moderately short. The limbs of the intestine are short, reaching the level of the middle of the testes. That on the right, running ventral to the ovary, ends between it and the right testes.

The *excretory vesicle* (Fig.77) is V-shaped, with wide limbs which stretch forward, and end just behind the testes; the pore opens at the posterior end, and is exactly terminal.

The testes are comparatively large, 0.43 mm. long, by 0.193 mm. broad, more or less oval in form. They lie right up to the lateral edges of the body (Fig.77), and just in front of the ventral sucker. The vasa deferentia run straight inwards, and join the vesicula seminalis, which is a coiled, wide tube lying in the base of the cirrus-sac. The cirrus-sac, which lies altogether in front of the ventral sucker, and mainly in front of the testes, is a large, somewhat S-shaped structure, wide behind, but narrowed in front; there is a conspicuous pars prostatica, and well-marked ejaculatory duct and penis. The common genital opening lies on the lateral edge of the body, alongside the oral sucker (Fig.78).

The *ovary* is pear-shaped, with the narrow end directed backwards; it lies on the right side, partly in front of the right testis, but more dorsal and nearer the middle longitudinal line of the body. The oviduct is given off from the narrow end, and runs directly backwards: very soon it widens slightly to form the ootype, and it is here surrounded by a thick mass of the large cells forming the shell-gland; and at this level, about opposite the middle of the testes, a comparatively large receptaculum seminis and Laurer's canal are given off. Laurer's canal is short and narrow, and opens on the dorsal surface, and opposite the anterior edge of the

ventral sucker. The female duct now runs backwards, near the middle longitudinal line of the body, forming a loop, the receptaculum seminis uterinum (Fig.79), filled with sperms, and reaching a length of one-third that of the body. From this point, the uterus proceeds in a number of loops, which fill up, first, the right side of the body behind the testes, then cross over near the ventral sucker and fill up the left side in the same extent, the metraterm finally running alongside the cirrus-sac to the genital chamber.

The *follicles* of the vitelline glands are few in number, and rather large in size, more or less pear-shaped. The right and left groups are connected by a continuous band of follicles, crossing the body in a dorsal position. There are about 10 on each side. The two groups are situated at the sides of the pharynx and œsophagus, and anterior and dorsal to the intestinal limbs.

The *eggs*, which fill up the numerous loops of the uterus, are in vast numbers. They are light brown in colour at first, becoming dark brown by the time they reach the distal end of the uterus. They are small in size, 0.0236 mm. long, by 0.0117 mm. broad; in shape they are almost a flattened ellipse, with the long sides approximately parallel to one another.

In its affinities, this Australian species appears to stand nearest to the two Asiatic species, *Pleurogenes gastroporus* Lühe, and *P. sphaericus* Klein; being, perhaps, nearest of all to the latter. While agreeing with each of these species in the relative positions of the testes and cirrus-sac, and in having the genital pore on a level with the oral sucker, our species further agrees with *P. sphaericus* in the position of the excretory pore, in having the testes alongside the ends of the intestinal limbs and in front of the ventral sucker, and in the disposition of the uterine loops; differing in these respects from *P. gastroporus*, which has the excretory pore quite ventral, and the testes behind the ends of the intestinal limbs and alongside the ventral sucker. *P. freycineti*, in the relatively smaller ventral sucker, differs from *P. sphaericus* (as indeed from all other species of the genus) in the small size of the

eggs, and in the spininess of the body, continued with hardly any diminution to the extreme posterior end of the body.

PLEUROGENES SOLUS, sp.n.

(Figure 17.)

*Diagnosis.*—Body oval, narrower at the anterior end, 0.815 mm. long, by 0.49 mm. broad; oral sucker subterminal, 0.111 mm. in diameter, ventral sucker *behind the middle* of the body, 0.121 mm. in diameter; *ratio of oral to ventral sucker*, 11:12. Skin spiny. Pharynx very well developed, globular, 0.051 mm. in diameter; œsophagus with the intestinal limbs reaching to the anterior edge of the ventral sucker. Excretory vesicle large, V-shaped, pore terminal. Testes small (0.098 mm. in diameter), round, symmetrically placed on either side of the ventral sucker; ovary oval (0.098 × 0.049 mm.), in front of and slightly internal to the right testis. Cirrus-sac large, 0.245 mm. long, S-shaped, entirely in front of ventral sucker; genital opening on a level with the pharynx. *Vitelline glands oval or pear-shaped, large* (0.058 × 0.028 mm.), *few* (nine on each side). Coils of the uterus as in *P. freycineti*, without a second loop crossing from one side to the other. Eggs yellow, elliptical, *very small*, 0.020 × 0.010 mm.

Host, *Hyla aurea*, in the intestine.

Type-specimen in the Australian Museum, Sydney, No. W.345.

This little species was found in the intestine of *Hyla aurea*, along with a large number of specimens of *Dolichosaccus*. Out of the hundreds of this kind of frog examined, only one single specimen of *Pleurogenes* was found. This specimen was fixed in sublimate acetic, kept flat by the vaseline-method, stained with borax-carminé, and mounted whole. It is obviously a distinct species from *Pleurogenes freycineti*, with such well-marked specific characters as, (i.) an entirely different relation in the size of the suckers, the ventral being larger in *P. solus*, while in *P. freycineti* it is the oral sucker which is larger than the ventral; (ii.) the comparatively longer intestinal limbs in *P. solus*; (iii.) the very much smaller gonads; (iv.) the more posterior position of the testes in relation to the ventral sucker; (v.) the more posterior position of the genital aperture; and (vi.) the smaller eggs.

The body is very similar in shape to *P. freycineti*, but much smaller; only about half the size. While the spines of *P. solus* are much smaller, their arrangement is very similar to that found in *P. freycineti*.\*

Literature referred to.—38, 48, 60, 66, 91.

#### SECTION V.

##### *Egg-formation and the function of Laurer's Canal.*

While studying living specimens of *Dolichosaccus trypherus* mounted in normal saline solution, I noticed the process of egg-formation going on, and, under the high power, was able to follow out many of the details. The ripe ova leave the ovary generally (and apparently normally) one at a time. Lying for a short time at the entrance of the oviduct, it is suddenly expelled from the ovary, entering the oviduct with a little gush, and almost immediately finds itself in the fertilization-space, where it is at once vigorously attacked by a large number of spermatozoa which completely surround it. This fertilization-space, which, as already described (p.312) is of great size, is generally filled with very lively spermatozoa, and fertilization takes place in that part opposite to its opening into the oviduct (Fig.5). For a few seconds, there is a very energetic turmoil, when, suddenly (one apparently having entered the ovum, though this could not be seen owing to the dense mass of sperm surrounding it) the spermatozoa leave the ovum, and at once enter Laurer's canal, down which they move with extraordinary energy. The Laurer's canal in this species is a very long one, pursuing a tortuous course right down to the level of the anterior testis, in which region it opens on the dorsal surface. The flight of the sperms seems to be influenced by the pressure of the surrounding tissues on the Laurer's canal, for here and there their onward rush is checked, and a wave of backward movement occurs for a moment; but they soon move onward again, and finally rush out through the opening of the canal into the surrounding salt solution.

\* Later, twelve additional specimens were found and placed at my disposal by Mr. E. A. Briggs, Assistant Zoologist to the Australian Museum.

In the meantime, the fertilized ovum has entered the ootype, and moves towards the entrance of the yolk-duct. Mostly the ovum moves up to that point and away again several times, but, finally, a number of yolk-cells (generally four) enter the ootype, and at once become fastened to one side of the ovum, so that this cell now forms the apex of a little group of five cells. The walls of the ootype now undergo movements which roll the forming egg about, as one might roll a ball of putty between the hands. In this way, the egg becomes shaped, and a shell appears. This forming shell is, at first, so transparent as apparently to be invisible, for when I have first been able to see it, it has already attained some thickness, and gradually becoming less transparent, it thus becomes more easily discernible. Unfortunately, I have not yet been able definitely to observe the shell being actually formed out of those globules in the yolk-cells, which Goldschmidt and others state(26) furnish the material of the shell; but I am convinced that I can confirm Goldschmidt's observations, for the reaction of the newly formed shell and of the globules in the yolk-cells to stains like safranin, for instance, which gives the most favourable results in this regard, is identical, while there is nothing in the "shell-glands" that becomes stained in the same way.

The shell having become formed, the egg is passed on to the uterus, and another oosperm takes its place in the ootype.

From the foregoing, it will be seen that, in this species at least, the function of Laurer's canal is to get rid of the sperms that have tried and failed to effect an act of fertilization, as contended in general by Looss(47, 48), Benham(2, p.88), Goto(27, p.174). It may also get rid of unused yolk-material, for, though I have not actually seen any in the canal itself, I have seen, near the opening of the canal on the exterior, little accumulations of objects that look like pieces of broken-up yolk-cells. From the study of another trematode, *Distomum pristiophori*,(32), I was formerly of opinion that Laurer's canal functioned as a vagina, for this canal, in that worm, was fairly wide, and possessed well-developed muscular walls, and, furthermore, was filled with sperms. It could quite well be, of course, that these sperms were

passing out, and it now appears that I was formerly led to an erroneous conclusion by a wrong inference from the presence of the muscular tissue in the walls and the size of the canal. In any case, in most of the worms studied in this paper, the metraterm is of such a form and structure that, taken in conjunction with the form of the penis in the same specimen, and with what I have seen of the function of Laurer's canal in *Dolichosaccus*, I think there can be no doubt that this metraterm is the real vagina.

Notwithstanding this, Cohn(12, 14) seems to have definitely observed one or more cases of actual copulation through Laurer's canal. I think, however, the instances mentioned by him must be looked upon as abnormal cases.

Literature—2,4, 12, 14, 22, 26, 27, 29, 30, 35, 37, 44, 45, 47, 48, 92.

#### SECTION VI.

*General Conclusions.*—A consideration of the trematodes described above, from Australian frogs, affords a striking illustration of the tendency of Helminths to occur in faunal groups. That is to say, that the Helminths found parasitic in any particular class of host, in a definite zoogeographical region, find their nearest relatives, not in that region in which they themselves occur, but in the same class of host living in other zoogeographical regions. Four such groups from Amphibia are now more or less extensively known, viz., European, North American, Asiatic, and Australian. In each region, we find, in hosts of this class, trematodes pretty closely corresponding with representatives in the other regions. The group in the European region, as the longest known and most extensively investigated, shows the greatest number of genera; in point of numbers, the American group, to which a good deal of attention has been given of late years by Stafford and others, follows closely on the European. The Australian and Asiatic groups show smaller numbers, partly, perhaps, because less completely worked up. The facts present seem to indicate that, in Asia and Australia, at least, the number of representatives may be further more or less extensively added to by subsequent investigations. Besides the frogs, other classes



of hosts, as mammals, birds, reptiles, show faunal groups of parasites with relations analogous to those exhibited by the group from frogs, as small collections of parasites in my possession obtained from hosts of the classes mentioned, go to show. But for the present, I must confine my remarks to the groups from the frogs. *Opisthioglyphe endoloba*, occurring in the intestine of European frogs, is represented in North America by *Glypthelminis quieta*, and in Australia by *Dolichosaccus* spp., which also live in the proximal part of the intestine of their hosts. The three European species of *Pneumonæces*, found in the lungs, are represented in America by no less than six species (*P. longiplexus* Stafford, *P. breviplexus* Staff., *P. varioplexus* Staff., *P. similiplexus* Staff., *P. medioplexus* Staff., and *P. complexus* Seeley), while they are represented in Asia by *Pneumonæces capyristes* Klein, and in Australia by *P. australis*. The *Gorgoderine*, represented in European frogs by the two genera, *Gorgodera* and *Gorgoderina*, comprising between them, according to Ssinitzin(73), five separate species, all living in the bladder of frogs, are represented in America by four species of *Gorgoderina* and one of *Gorgodera*. No species of *Gorgodera* has yet been described from Asia, but the genus is represented by one species in Australia. The European *Brachycolium crassicolle* R., found in the intestine, is represented in America by *B. hospitale*, in Australia by three species of *Mesocolium*, and in Asia by *M. sociale*. The *Pleurogenetine*, occurring in the anterior part of the intestine, are represented in Europe by three genera (*Prosotocus*, *Pleurogenes*, and *Brandesia*), comprising, between them, seven species; in America by *Loxogenes arcanum*, in Asia by *Pleurogenes gastroporus* and *P. sphericus*, and in Australia by *P. freycineti* and *P. solus*. *Halipegus ovocaudatus*, occurring in the buccal cavity of European frogs, is represented in North America by *H. occidualis*, in South America by *H. dubius*(Klein, 38, p.68), and in Asia by *H. longispina*, all living in similar situations; but, up to the present, no representatives of this fluke have been found in Australia. *Diplodiscus subclavatus*, in the rectum of European frogs, is represented in America by *D. temperatus*, and in Australia by two species of *Diplodiscus*. The European *Polystomum integer-*

*rinum*, in the bladder of frogs, does not seem to be represented in America in frogs, but three species (*P. coronatum* Leidy, *P. hassalli* Goto, and *P. oblongum* R. Wright) occur there in the bladders of *Chelonians*; while in Australia, this heterocotylean genus is represented by one species in the bladder of frogs. No representatives of *Diplodiscus* nor *Polystomum* have yet been described from Asia. The American *Cephalogonimus americanus*, in the intestine of frogs, may be represented in the Old World by *C. lenoiri* Poir. The genus *Gaueo*, described by Klein from the Indian *Rana hexadactyla*(38), seems to stand alone, unrepresented in any other region.

It is a remarkable fact that, of the six species of flukes described from frogs inhabiting southern Asia, four of them appear to find their nearest relatives in flukes from Australian frogs. *Mesocaulium sociale* Lühe, is certainly more closely related to the Australian species of *Mesocaulium* than to *Brachycaulium crassicolle* R., its European, or *B. hospitale* Staff., its American representative. *Pneumoneces capyristes* Klein, has been shown above (Section iv.) to be more nearly related to *P. australis* than to any other of the European or American species of this genus; and the Asiatic *Pleurogenes gastroporus* and *P. sphaericus*, and the Australian *P. freycineti* and *P. solus* have likewise been shown to be more nearly related to one another than any of them are to the American or European pleurogenetines.

I think the Asiatic frog-flukes, so far as they are known, are more nearly related to the European than are the Australian, standing, as it were, intermediate between the two latter groups. The American frog-flukes, on the other hand, many of which have evolved into distinct genera, are not so nearly related in their structure to the European as are the Asiatic. And, in addition to this, the American genera, generally speaking, contain more species than the same genera in Asia and Australia, and this may be taken to indicate that the American frogs, with their flukes, have been longer separated from the parent-stock.

The great similarity of the four groups of flukes of frogs found in the four regions mentioned, points to the fact, I think, that the flukes are a very old group of animals, and existed in the ances-

tors of present-day frogs a very long time ago, when their distribution was much less extensive than it is to-day. The mutual relationships of these groups of trematodes support the view that the Anura originated somewhere about the centre of the Palæarctic region, and migrated both westwards and south-westwards. They may have reached the western portion of the Boreal land-mass, existing right across from Asia to North America, in early Tertiary times, or they may have made their way westward in Pliocene times when a considerable migration of vertebrates westwards is known to have taken place(34). The Australian forms must have found their way down here before the separation of the Australian continent from south-eastern Asia, a separation which is generally supposed to have taken place somewhere about late Cretaceous or Eocene times. The greater diversity of the North American frog-trematodes would seem to indicate that they have been longer separated from the parent-stock than the Asiatic and Australian forms, so that the America-wards migration probably took place in the earlier of the two periods suggested.

In view of the probable land-connection between Australia and South America through the Antaretic, a connexion which is supported by a good deal of biological evidence, it is unfortunate that practically nothing seems to be known about the frog-trematodes of South America. I can find only two indirect references to any such trematodes, viz., in Braun(4, p.906), and Klein(38, p.68). A pretty close similarity has, however, been shown by Zschokke(106) to exist between some cestode-parasites of South American and Australian marsupials.

The close similarity existing between the respective representatives in the four groups of frog-trematodes in question here reminds us that the trematodes, owing to the conditions under which their lives are passed, have probably evolved much more slowly than their hosts, for the amphibian ancestors of our present-day groups, at the time of their dispersal, must have been considerably different from their representatives now living.

In conclusion, I wish to thank Professor Haswell, in whose laboratories this work has been carried out, for much valuable

help and encouragement during its progress, as well as to express my indebtedness to Mr. Walter Graham for much help in searching the frogs for their trematode parasites.

## SECTION vii.

List of frogs with the trematode-parasites occurring in them, described in this paper.

## LIMNODYNASTES PERONII.

- Pneumonæces australis*, in the lungs.  
*Dolichosaccus trypherus*, in the duodenum,  
*Brachysaccus anartius*, in the rectum.  
*Diplodiscus megalochrus*, in the rectum.  
*Gorgodera australiensis*, in the bladder.

## LIMNODYNASTES TASMANIENSIS.

- Diplodiscus microchrus*, in the rectum.

## LIMNODYNASTES DORSALIS.

- Dolichosaccus ischyrys*, in the duodenum.

## HYLA CŒRULEA.

- Mesocœlium mesembrinum*, in the duodenum.  
*Dolichosaccus ischyrys*, in the intestine.  
*Brachysaccus symmetricus*, in the rectum.

## HYLA PHYLLOCHROA.

- Polystomum bulliense*, in the bladder.

## HYLA CITROPUS.

- Mesocœlium oligoon*, in the duodenum.

## HYLA EWINGII.

- Mesocœlium megaloon*, in the duodenum.  
*Diplodiscus microchrus*, in the rectum.

## HYLA AUREA.

- Pneumonæces australis*, in the lungs.  
*Dolichosaccus trypherus*, in the duodenum.

*Pleurogenes solus*, in the intestine.

*Brachysaccus anartius*, in the intestine and rectum.

*Diplodiscus megalochrus*, in the rectum.

*Gorgodera australiensis*, in the bladder.

#### HYLA LESUEURII.

*Polystomum bulliense*, in the bladder.

#### HYLA FREYCINETI.

*Dolichosaccus diamesus*, in the stomach.

*Pleurogenes freycineti*, in the duodenum.

### SECTION viii.

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EXPLANATION OF THE FIGURES.

The drawings, which were made by Mr. F. W. Atkins, of Sydney, were done with the help of the camera lucida. The microphotographs are from “untouched-up” negatives of sections and whole-mounts; most of them are by Mr. Louis Schaeffer, of the Department of Anatomy, University of Sydney.

*Reference letters.*

*C.s.*, Cirrus-sac—*Eg.*, Egg—*Ej. d.*, Ejaculatory duct—*E.*, Excretory vessels—*Ex.p.*, Excretory pore—*Ex.v.*, Excretory vesicle—*F.s.*, Fertilization-space—*F.c.*, Flame cells—*G.p.*, Genital pore—*G.c.*, Genital chamber—*Int.*, Intestinal limbs—*L.c.*, Laurer’s canal—*M.o.*, Mouth opening—*Mtm.*, Metraterm—*Mus.*, *Muscle*

fibres—*N.*, Nervous system—*Es.*, Oesophagus—*O.s.*, Oral sucker—*O.*, Ovary—*O.d.*, Oviduct—*Oot.*, Ootype—*P.*, Penis—*Ph.*, Pharynx—*P.ph.*, Praepharynx—*Pr.*, Prostate—*Ps.*, Receptaculum seminis—*R.s.u.*, Receptaculum seminis uterinum—*S.g.*, "Shell-glands"—*T.*, Testis—*Ut.*, Uterus—*Vag.*, Vagina or Metraterm—*V.d.*, Vas deferens—*V.s.*, Vesicula seminalis—*V.sk.*, Ventral sucker—*Y.d.*, Yolk-duct—*Y.g.*, Yolk-glands—*Y.r.*, Yolk-reservoir.

Figures 1-17 are from drawings; 18-80 from microphotographs. All the figures are from the ventral surface, except where stated otherwise.

Fig.1.—*Polystomum bulliense* ( $\times 40$ ). *G.i.c.*, genito-intestinal canal—*Hk.*, large hooks on disc—*Vag.p.*, vaginal papillæ—Fig.1a., one of the large hooks ( $\times 290$ )—Fig.1b., one of the larval hooks ( $\times 290$ ).

Fig.2.—Diagram of the genital organs of *P. bulliense*. *G.i.c.*, genito-intestinal canal—*Vag.p.*, vaginal papillæ—*Vag.r.*, sperm-reservoir at the beginning of the vaginal canal.

Fig.3.—*Diplodiscus megalochrus* ( $\times 37$ ). *B.d.*, buccal diverticulum—*c.d.*, central disc of *P.sk.*, posterior sucker.

Fig.4.—*Dolichosaccus trypherus* ( $\times 33$ ).

Fig.5.—Diagram of the female organs of *D. trypherus*.

Fig.6.—Excretory system of *D. trypherus* ( $\times 61$ ).

Fig.7.—*Dolichosaccus ischyryus* ( $\times 43$ ).

Fig.8.—*Dolichosaccus diamesus* ( $\times 61$ ).

Fig.9.—*Brachysaccus anartius* ( $\times 43$ ).

Fig.10.—*Brachysaccus symmetricus* ( $\times 96$ ).

Fig.11.—*Pneumonæces australis* ( $\times 84$ ).

Fig.12.—*Gorgodera australiensis* ( $\times 84$ ).

Fig.13.—*Mesocelium mcsebrinum* ( $\times 33$ ).

Fig.14.—*Mesocelium oligoon* ( $\times 60$ ).

Fig.15.—*Mesocelium megaloon* ( $\times 84$ ).

Fig.16.—*Pleurogenes freycineti* ( $\times 60$ ).

Fig.17.—*Pleurogenes solus* ( $\times 60$ ).

Fig.18.—L.S., *Polystomum* ( $\times 130$ ), passing through the middle of a posterior sucker, showing the connective tissue plug, retractor muscle, etc.

Fig.19.—L.S., *Polystomum* ( $\times 210$ ), through the oral sucker.

Fig.20.—T.S., *Polystomum* ( $\times 255$ ), through pharynx, buccal pocket, and cerebral ganglia.

Fig.21.—T.S., *Polystomum* ( $\times 160$ ), through excretory vesicles.

Fig.22.—T.S., *Polystomum* ( $\times 130$ ), through ovary, uterus, vas deferens.

- Fig.23.—T.S., *Polystomum* ( $\times 370$ ), through vaginal openings and sperm-reservoir of the vaginal canal.
- Fig.24.—T.S., *Polystomum* ( $\times 130$ ), through testis, and descending and ascending loops of the uterus.
- Fig.25.—L.S., *Polystomum* ( $\times 40$ ), showing the relations of the distended uterus and the yolk-glands.
- Fig.26.—H.S., *Polystomum* ( $\times 30$ ), showing the relations of the genital organs, alimentary canal, etc.
- Fig.27.—H.S., *Polystomum* ( $\times 175$ ), through the opening of the genito-intestinal canal into the intestine.
- Fig.28.—L.S., *Polystomum* ( $\times 130$ ), through the vaginal papillæ.
- Fig.29.—*Diplodiscus megalochrus* ( $\times 25$ ); photograph of a whole-mount.
- Fig.30.—*Diplodiscus microchrus* ( $\times 37$ ); photograph of a whole-mount.
- Fig.31.—H.S., *Diplodiscus megalochrus* ( $\times 35$ ), showing the structure of the posterior sucker.
- Figs.32-33. L.S. and T.S., respectively, through the posterior sucker of *Diplodiscus* ( $\times 65$ ), showing the connective tissue plug of large parenchyma-cells, etc.
- Fig. 34.—H.S., *Diplodiscus megalochrus* ( $\times 65$ ), through pharynx and intestine.
- Fig.35.—H.S., *Diplodiscus megalochrus* ( $\times 300$ ), through pharynx, showing the muscular layers in its walls.
- Fig.36.—T.S., through pharynx of same, showing the numerous layers of circular muscle-fibres ( $\times 370$ ).
- Fig.37.—H.S., *Diplodiscus megalochrus* ( $\times 110$ ), through oral sucker and its diverticula.
- Fig.38.—T.S., *Diplodiscus megalochrus* ( $\times 100$ ), through the buccal diverticula and œsophagus.
- Fig.39.—T.S., *Diplodiscus megalochrus* ( $\times 85$ ), through the genital opening.
- Fig.40.—T.S., *Diplodiscus megalochrus* ( $\times 220$ ), through vagina and cirrus-sac.
- Fig.41.—T.S., *Diplodiscus megalochrus* ( $\times 90$ ), through testis.
- Fig.42.—T.S., *Diplodiscus megalochrus* ( $\times 90$ ), through ovary, oviduct, and shell-gland.
- Fig.43.—L.S., *Dolichosaccus trypherus* ( $\times 80$ ), showing præ-pharynx, Laurer's canal, etc.
- Fig.44.—L.S., *Dolichosaccus trypherus* ( $\times 160$ ), through Laurer's canal, etc.

- Fig.45.—*Dolichosaccus trypherus* ( $\times 27$ ); photograph of whole-mount.
- Fig.46.—*Dolichosaccus ischyryus* ( $\times 32$ ); photograph of whole-mount.
- Fig.47.—T.S., *Dolichosaccus trypherus* ( $\times 170$ ), through genital opening, showing copulatory organ, etc.
- Fig.48.—L.S., *Dolichosaccus ischyryus* ( $\times 55$ ), showing the structure of the vesicula seminalis.
- Fig.49.—L.S., *Dolichosaccus ischyryus* ( $\times 55$ ), showing the very large excretory vesicle, Laurer's canal, etc.
- Fig.50.—T.S., *Dolichosaccus ischyryus* ( $\times 170$ ), behind the testes, showing excretory vesicle, distribution of the yolk-glands, etc.
- Fig.50a.—H.S., *Dolichosaccus ischyryus* ( $\times 305$ ), near the surface, showing the form and arrangement of the spines.
- Fig.51.—*Brachysaccus anartius* ( $\times 33$ ); photograph of whole-mount.
- Fig.52.—H.S., *Brachysaccus anartius* ( $\times 40$ ), showing præpharynx, etc.
- Fig.53.—H.S., *Brachysaccus anartius* ( $\times 140$ ), showing Laurer's canal opening near the posterior end.
- Fig.54.—H.S., *Brachysaccus anartius* ( $\times 57$ ), showing the Y-shaped excretory vesicle.
- Fig.55.—H.S., *Brachysaccus anartius* ( $\times 370$ ), showing spines.
- Fig.56.—*Pneumonæces australis* ( $\times 25$ ); photograph of a whole-mount, showing the typical arrangement of the yolk-glands.
- Fig. 57.—Photograph of another whole-mount, showing the general anatomy ( $\times 22$ ).
- Fig.58.—L.S., *Pneumonæces australis* ( $\times 82$ ), showing penis, etc.
- Fig.59.—L.S., *Pneumonæces australis* ( $\times 37$ ), showing the elongated vesicula seminalis.
- Fig.60.—T.S., *Pneumonæces australis* ( $\times 100$ ), showing the coiled arrangement of the vesicula seminalis near its anterior end.
- Fig.61.—T.S., *Pneumonæces australis* ( $\times 80$ ), showing ovary, receptaculum seminis, etc.
- Fig.62.—*Pneumonæces australis* ( $\times 70$ ), showing the arrangement of the uterine loops behind the testes, position of the yolk-glands, etc.
- Fig.63.—*Gorgodera australiensis* ( $\times 30$ ); whole-mount.
- Fig.64.—H.S., *Gorgodera australiensis*, showing arrangement of uterine loops.

- Fig.65.—L.S., *Gorgodera australiensis* ( $\times 180$ ), showing vesicula seminalis, ejaculatory duct, common genital chamber, metraterm.
- Fig.66.—T.S., *Gorgodera australiensis* ( $\times 350$ ), showing yolk-glands and duct, oviduct, ootype, Laurer's canal.
- Fig.67.—T.S., *Gorgodera australiensis*, showing the arrangement of the uterine loops behind the testes.
- Fig.68.—T.S., *Gorgodera australiensis* ( $\times 120$ ), through ovary, oviduct, receptaculum seminis uterinum, etc.
- Fig.69.—*Mesocœlium mesembrinum* ( $\times 40$ ), whole-mount with the uterus only moderately filled with eggs, so that the general anatomy is more or less visible.
- Fig.70.—L.S., *Mesocœlium mesembrinum* ( $\times 27$ ), through the excretory vesicle.
- Fig.71.—T.S., *Mesocœlium mesembrinum* ( $\times 200$ ), passing through the bifurcated anterior end of the excretory vesicle.
- Fig.72.—T.S., *Mesocœlium mesembrinum* ( $\times 145$ ), passing through the copulatory organs and the œsophagus.
- Fig.73.—T.S., *Mesocœlium mesembrinum* ( $\times 200$ ), through the vagina, ejaculatory duct, etc.
- Fig.74.—T.S., *Mesocœlium mesembrinum* ( $\times 200$ ), through the cirrus-sac, vagina, testes, vas deferens, etc.
- Fig.75.—T.S., *Mesocœlium mesembrinum* ( $\times 200$ ), through Laurer's canal, etc.
- Fig.76.—T.S., *Mesocœlium mesembrinum* ( $\times 200$ ), through ovary, ootype, shell-gland, etc.
- Fig.77.—H.S., *Pleurogenes freycineti* ( $\times 105$ ), showing the V-shaped excretory vesicle.
- Fig.78.—H.S., *Pleurogenes freycineti* ( $\times 115$ ), showing the position of the genital opening.
- Fig.79.—*Pleurogenes freycineti* ( $\times 70$ ), whole-mount, showing the arrangement of the uterine loops.
- Fig.80.—*Pleurogenes freycineti* ( $\times 140$ ), near the surface, showing the form and arrangement of the spines or scales.

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POSTSCRIPT [*added 9th November, 1912*].—On November 1st, I obtained from the duodenum of two specimens of *Hyla citropus*, collected at Springwood, ninety-four specimens of *Mesocœlium oligoon*; up to this time, I had obtained only two specimens of this worm.